Conflict in the Communal Nest: Investigating Female Competition in House Mice

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

Lisa Bottell

February 2013

Contents

List of figures

List of tables

Acknowledgements

First and foremost I would like to thank my supervisors Paula Stockley and Jane Hurst for all of the guidance and expertise they have shared with me over the past four years. I am particularly grateful for their support and patience during the break from my research. It has been wonderful to be supervised by two incredibly enthusiastic and dedicated experts, and I really could not have achieved this without their support.

I would like to thank all members of the Mammalian Behaviour and Evolution group, past and present, for fascinating journal clubs, lab meetings and helpful comments on my work over the past four years. Particular thanks go to John Waters, Rick Humphries, Amanda Davidson, Rachel Spencer, Sue Jopson and Felicity Fair for technical help and animal care; to Sarah Roberts for always having the patience to teach me a diverse range of skills that have been essential to my PhD; to Mike Garratt for help and advice on setting up the enclosure room and running testosterone assays; to Amanda Davidson for running all the mass spectrometry and providing advice for biochemical techniques; finally to Andrew Holmes for proof reading my work at a time when he was trying to complete his own thesis (I'm so sorry)!

I am grateful for the financial support provided by the UK Biotechnology and Biological Science Research Council (BBSRC) for funding this research. Additional thanks also go to the Association for the Study of Animal Behaviour (ASAB) who provided travel grants towards attending conferences. I also wish to thank my undergraduate supervisor Paul Cunningham and programme leader Penny Oakland for the inspiration and encouragement they gave me to undertake a PhD.

To Andrew, Fliss, Jane and Sarah, thank you for being such wonderful friends. Each one of you has helped me far more than you know and I feel incredibly lucky to have such good friends.

Finally I would like to thank my wonderful family for all of their love and encouragement during the most difficult five years of our lives; to my amazing Mum for being such an inspiration and always encouraging me to follow my heart, to Nan for her love and constant worrying (perhaps you'll worry less about me now!), to Michael and James for always making me laugh and believing in me, and to Roo because I really couldn't have done this without your unfaltering belief, encouragement and love.

This thesis is dedicated to Charles Bottell who encouraged his little girl to always aim high and believed that she could achieve anything she set her heart on.

I hope I made you proud Daddy…

Thesis abstract

Female-female competition has been relatively overlooked in favour of male-male competition for mates, but it can also have important reproductive consequences. There are an increasing number of studies describing conditions where females compete to obtain breeding rank, gain access to or control resources or actively defend young. Communally breeding females are thought to be relatively egalitarian, sharing the cost of parental care with other females. Hence little attention has been paid to the potential for competition in such breeding systems, despite evidence of aggression and reproductive suppression between females. This thesis therefore explores the extent of competitive behaviours between female wild house mice (*Mus musculus domesticus*), a species with communal care of young, and investigates the physiological effects of competition and its consequences for breeding success and reproductive output.

I examined the effects of age and other characteristics that may predict the degree of female competition. I identified that body mass, relative age of social partners, urinary testosterone concentration and reproductive experience were all useful predictors for the amount of competitive behaviour observed between female pairs. Following competitive female interaction I found that urinary testosterone and protein output increased, but there was no significant change in body mass and no significant effect on oestrus cycle length. Older females (> 12 months) with competitive experience had larger adrenal glands compared to females previously housed with their sisters, suggesting a possible stress response to competitive interaction. There was also evidence that competitively housed females had enlarged clitoral glands, which may play a role in signalling social status.

As female house mice were found to compete and assume social ranks, I investigated the impact of female social status on male mate choice and mating behaviour. There was no evidence of significant male preference for more or less competitive females prior to or after competitive interaction in a choice test with restricted access to females or when presented with female odours. To investigate breeding behaviour I introduced female pairs to a male in semi-naturalistic enclosures, filming continuously over a four day period to examine mating attempts and female behaviour. Interestingly males mounted less competitive females either exclusively or preferentially during the test, with a small number of competitive females interrupting mating behaviour between their social partners

and the male. Therefore males may prefer female partners that are less likely to act aggressively towards their advances.

The effect of female competition on reproductive success was examined by comparing breeding success of subjects under solitary and communal breeding conditions. Despite the prediction that reproductive success increases for secondary litters in house mice, reproductive output was significantly reduced for more and less competitive females in the communal nest compared to previous output in a solitary nest. This finding illustrates the negative impact of competition on reproductive success. Females that gave birth first in communal nests also had significantly fewer pups present on post natal day one compared to females that gave birth second. Interestingly female offspring of more competitive females in this experiment went on to produce larger litters on average than females born to less competitive females. Litters were also likely to be male biased if females had been reared in a competitive environment rather than a solitary nest, suggesting that competitive ability and rearing environments can both influence reproductive success for offspring.

These results, together with evidence in the literature, suggest that competition does occur between communally breeding females, and that reproductive success can be affected as a result. However competition between communal females may be less intense than between females in cooperative systems, where reproductive skew is biased towards one or two individual females in a group. Using a comparative analysis I found that cooperatively breeding species had increased reproductive output compared to other polytocous species, which is likely to be influenced by the presence of non-breeding helpers in the nest site. Cooperative species also had decreased inter-litter intervals compared to non-cooperative species, as well as a reduction in lactation length and protein content of milk. Communal species were found to have increased offspring growth and reduced sexual size dimorphism, suggesting that competition between females may have resulted in selection for increased female body mass.

Together these results illustrate the significance of female competition in wild house mice, with consequences for mating behaviour and reproductive success, as well as the evolutionary implications of female-female competition in mammalian species residing in communal breeding systems.

Chapter 1 Introduction – Female competition in social species

1.1 Chapter overview

Competition between females (hereafter female competition) has been relatively overlooked in favour of male-male competition for mates, but it can also have important reproductive consequences. However, there are an increasing number of studies describing conditions where females compete to obtain breeding rank or to gain access to reproductive resources, and a wide range of competitive strategies are also adopted to defend offspring. Communally breeding females are generally thought to be relatively egalitarian, sharing the cost of parental care with other females in the nest. Hence little attention has been paid to the potential for competition in such breeding systems, despite evidence of aggression and reproductive suppression between females, as well as infanticidal behaviour. This introduction explores the extent of competition between females in a diverse range of species, with a particular reference to species exhibiting cooperative care. Finally I introduce the study species of this thesis, wild house mice (*Mus musculus domesticus*).

1.2 Female competition

Female competition has received relatively little attention compared to male competition, due to the relative intensity of competition for breeding partners in the two sexes (Clutton-Brock, 2009b; Cunningham & Birkhead, 1998; Darwin, 1871; Trivers, 1972). The potential rate at which males and females can reproduce is restricted by differences between the sexes in parental investment (Bateman, 1948; Trivers, 1972). As the cost of producing gametes and rearing offspring is typically greater for females, males are considered to invest less in reproduction and can therefore reproduce at a faster rate, resulting in a male biased operational sex ratio (i.e. the ratio of fertilizable females available to sexually active males) (Emlen & Oring, 1977; Trivers, 1972). Sexual selection is therefore expected to be stronger in males, favouring an ability to effectively compete with their rivals for mates and display their quality as breeding partners to females (Bateman, 1948; Clutton-Brock & Vincent, 1991; Clutton Brock & Parker, 1992; Darwin, 1871; Trivers, 1972). In some bird and mammal species however, females show also some development of secondary sexual characteristics, raising important questions about the evolutionary mechanisms responsible (Clutton-Brock, 2007; Clutton-Brock, 2009b; Isaac,

2005; Kraaijeveld *et al.*, 2007). Competitive patterns in female mammals are likely to change throughout the reproductive cycle, with females competing over mates when oestrus is synchronised, over food during gestation and lactation periods, and also for investment in offspring care during lactation (Huchard & Cowlishaw, 2011). Subsequent research has therefore highlighted the intensity of reproductive competition between females including the potential for females to develop secondary sexual characteristics in response to competition (see Clutton-Brock, 2007; Clutton-Brock, 2009b; Cunningham & Birkhead, 1998; Stockley & Bro-Jørgensen, 2011).

1.2.1 Competition for reproductive resources

Female reproductive success can be strongly influenced by access to resources necessary for health and survival, such as food and water (Emlen & Oring, 1977; van Noordwijk & van Schaik, 1987), for resources that decrease the chance of predation, such as sheltered breeding sites or assisted defence from the territorial male (Agrell *et al.*, 1998; Koskela *et al.*, 1997) and/or for assistance with offspring care (Clutton-Brock *et al.*, 2001b; Féron & Gouat, 2007; Taborsky, 1985). Competitive relationships between female mammals may be determined by a combination of ecological traits such as home range size, predation rates, food type and distribution (Isbell & Young, 2002). For example, scramble competition can occur between groups if travel distances to food sites are long (Isbell, 1991). Under these conditions, dominant females are more likely to gain access to high quality feeding sites compared to subordinate females, and foraging efficiency may be improved among high-ranking individuals as a result of supplanting others and receiving fewer interruptions during feeding (Clutton-Brock *et al.*, 2006; Vogel, 2005). Both environmental conditions and group size were found to be important for reproductive rate in a study on *Papio* species, as inter-birth intervals were longer at extreme high and low temperatures and also when group size was relatively small or large (Hill *et al.*, 2000). In larger groups of long-tailed macaques (*Macaca fascicularis*), high ranking females may also gain more central group positions, minimising the risk of predation and consequently improving survival (van Noordwijk & van Schaik, 1987). Dominance relationships may therefore form within female groups due to the fitness advantages attributed to gaining this position (e.g. Dunbar, 1988; King *et al.*, 2008; van Noordwijk & van Schaik, 1999).

Group living females sometimes form coalitions with their relatives against rival groups to defend resources that contribute to reproductive success; for example, when food resources

are clumped (Dunbar, 1988; Van Schaik, 1989). The habitat saturation hypothesis states that under limiting food and/or space, individuals are more likely to cooperate as the costs of dispersal are high (Getz *et al.*, 1992). Indeed, ecological conditions have recently been suggested as a driving force for the evolution of cooperative breeding in birds, as many cooperative species live in relatively harsh conditions (Jetz & Rubenstein, 2011). As a consequence of living in challenging conditions however, breeding sites could become crowded, resulting in increased competition not just for resources important for reproduction, but also for mates (Hayes, 2000; Jetz & Rubenstein, 2011; Koenig *et al.*, 1992).

1.2.2 Competition for mates

Females can be expected to compete for males when the operational sex ratio (OSR) is female biased (i.e. the potential reproductive rate of females is higher than that of males), or if high quality males are in short supply (Berglund *et al.*, 1993). Female competition for mates is therefore widespread in polyandrous birds where OSRs are female biased (Emlen & Oring, 1977). Paternal care also influences OSRs as male reproduction is constrained by investment in young; for example in the monogamous seahorse (*Hippocampus subelongatus*) males provide paternal care by brooding eggs, and therefore female reproductive success is influenced by outcomes of competitive interactions with rival females (Kvarnemo *et al.*, 2007). Female competition for mates among polygynous mammal species however is often disregarded, as males are usually the competing sex (Bebié & McElligott, 2006); although female mammals may compete for access to mates under conditions of sperm limitation, for the resources males can provide, or to prevent future resource competition for their own offspring (Stockley & Bro-Jørgensen, 2011).

Where females gain benefits from mating with multiple partners in a single breeding cycle, or the risk of not being fertilised is high, female competition for mates is increased (Clutton-Brock, 2009b; Stockley & Bro-Jørgensen, 2011). Oestrus synchronisation can lead to reductions in the male bias of OSRs and increase the rate at which males mate; consequently male sperm reserves can be depleted, compared to when oestrus is asynchronous (Emlen & Oring, 1977). Female topi (*Damaliscus lunatus*) aggressively compete with each other to mate with preferred males on central lek territories and avoid mating with sperm depleted males during peak periods of mating (Bro-Jorgensen, 2002). As topi have short oestrus periods lasting one day, females adopt multiple mating

strategies, mating with between four and twelve males during this time (Bro-Jorgensen, 2007). Females of other species are also thought to increase aggression during the mating season in response to sperm competition, such as ring-tailed lemurs (*Lemur catta*) (von Engelhard *et al.*, 2000). Females may also compete for mating opportunities with favoured males, for example in langurs (*Presbytis entellus*), females disrupt copulations between the group male and other females (Sommer & Rajpurohit, 1989). Conversely, lower-ranking western gorilla females (*Gorilla gorilla*) increase post-conceptive mating with the breeding male during periods of receptivity (Doran-Sheeny *et al.*, 2009). This strategy may serve to maintain male interest in the high-ranking female and potentially delay conception in other females (Doran-Sheeny *et al.*, 2009).

Many female mammals use specific cues to signal reproductive status and receptivity to sexually mature males; for example facial colouration in mandrills (*Mandrillus sphinx*) (Setchell *et al.*, 2006), copulatory calls during the fertile phase in Barbary macaques (*Macaca sylvanus*) (Semple & McComb, 2000), and scent cues in golden hamsters (*Mesocricetus auratus*) (DelBarco-Trillo *et al.*, 2009). Where males encounter females simultaneously, females may use these signals to compete with each other in order to attract a mate.

1.3 Male mate preferences

Mate choice occurs when individuals show a preference for mating with a particular category of partner, irrespective of mating success (Clutton-Brock & McAuliffe, 2009) and is predominately performed by females across a wide range of species (see Clutton-Brock & McAuliffe, 2009; Halliday, 1983). Male mate choice on the other hand, is thought to evolve when females are encountered simultaneously (Barry & Kokko, 2010). The availability of mates and capability to access breeding females is also important (Clutton-Brock & McAuliffe, 2009; Edward & Chapman, 2011). On encountering a sexually mature female, males can choose to reject or accept courting females; alternatively they may court particular females they are attracted to (Tudor & Morris, 2009). The reproductive consequences of mating with less preferred females have been shown to reduce reproductive output in house mice (Gowaty *et al.*, 2003) and therefore mate choice in males could be important for reproductive success.

Where female quality can affect reproductive success, males may use conspicuous signals of female quality to select a mate, such as size of pinnate leg scales in the cooperative cichlid fish (*Neolamprolagus pulcher*) (LeBas *et al.*, 2003), or body size in fruit flies (*Drosophilia melanogaster*) (Byrne & Rice, 2006). For example, males may also be able to select mates on the basis of mating history in vole species (*Microtus ochrogaster, Microtus montanus*) (Ferguson *et al.*, 1986), or length of time to prenuptial molting in hermit crabs (*Pagurus nigrofascia*) (Suzuki *et al.*, 2012) as this may increase the chance that copulation will be successful. Body mass is important for male mate choice in guppies (*Poecilia reticulate*) (Herdman *et al.*, 2004), whereas female age is important for other species; for example chimpanzee (*Pan troglodytes*) males prefer to court older females (Muller *et al.*, 2006). Conversely, in the Carolina anole (*Anolis carolinensis*) males prefer younger (and novel) mates (Orrell & Jenssen, 2002). Competitive females may also be more desirable mates to sire offspring with, particularly if females are able to defend offspring (Maestripieri & Alleva, 1991); however there may be a trade off if aggressive females are also more likely to perform infanticide against their own young (Palanza & Parmigiani, 1994).

1.4 Effects of female competitive rank on reproductive success

Female competitive strategies at the time of reproduction vary between avian and mammalian species due to the differences in gestation. Viviparity makes it more difficult for a single female to monopolise breeding as females need to compete with other conspecifics rather than defend a single nest site (Raihani & Clutton-Brock, 2010). In bird species such as ostriches (*Struthio camelus*), dominant females remove eggs laid by subordinate females without evicting them, reducing the potential for aggression between females (Bertram, 1979). However in some species, female aggression towards rivals can be so intense that it increases the potential to form monogamous pairings with a male (e.g. in the European starling *Sturnus vulgaris*) (Sandell & Smith, 1997). The majority of mammalian species exhibit female philopatry, resulting in highly related social groups (Greenwood, 1980). The strength of female competition is likely to be higher in species forming larger groups (Hodge *et al.*, 2008), resulting in maturing females competing with relatives from previous generations (Gerlach, 1990). If there are limited breeding opportunities within these groups, females may queue for reproduction and aggressively compete for rank position when it becomes available (Kokko & Johnstone, 1999).

Individuals close to the front of queue are therefore more likely to perform costly and risky behaviour, as they have the greatest chance of inheritance, and consequently more to lose (Cant, 2006; Cronin & Field, 2007).

The benefits of dominance rank may also transfer to offspring. For example in Rhesus macaques (*Macaca mulatta*) offspring of dominant females are more likely to survive than offspring born to subordinate females (Meikle & Vessey, 1988). The age in which daughters reach sexual maturity may also be influenced by the dominance rank of mothers, for example in chimpanzees (*Pan troglodytes*) (Pusey *et al.*, 1997). Maternal rank can also determine the dominance position of offspring born into the group, resulting in lifetime fitness benefits for offspring and increased inclusive fitness benefits for the mother, for example in gelada baboons (*Theropithecus gelada*) (Dunbar, 1980). In some cooperatively breeding species, subordinate females may be evicted from the group if they become pregnant, often resulting in reproductive failure (Bell *et al.*, 2012). In naked mole rats (*Heterocephalus glaber*), females are inhibited due to the presence of circulating hormones and limited aggression from the dominant female to ensure monopolisation of breeding (Faulkes & Abbott, 1997).

Reproductive skew describes the difference in reproductive output between group members, where a value of one represents a single female producing all offspring (e.g. in cooperatively breeding species) and a value of zero represents a condition where all females reproduce and produce an equal number of offspring (e.g. in some communally breeding species); however a skew of zero is most likely to occur in groups of related individuals, due to lower potential for competition (Konig, 2006). Values between zero and one are described as a median skew, occurring when relationships are relatively despotic, with decreasing relatedness and increasing group size (Cant & Johnstone, 1999; Konig, 2006). It may therefore be important for females living in highly competitive social conditions to obtain and maintain dominance rank, in order to increase the chances of successful reproduction (Bridge & Field, 2007; Clutton-Brock *et al.*, 1984; Hodge *et al.*, 2008; Pusey *et al.*, 1997).

1.5 Competitive traits in females

Body size and mass are generally correlated with strength in a diverse range of species, meaning larger, heavier individuals are more likely to win contests; examples can be found

in arachnids (Wells, 1988), fishes (Enquist *et al.*, 1990; Lindström, 1992), mammals (Clutton-Brock *et al.*, 1980) and reptiles (Zucker & Murray, 1996). Other traits can also influence competitive success, such as competitive experience, physiological state and weaponry (Arnott & Elwood, 2009). In this section I will provide examples of specific traits that have been shown to influence female competitive ability across a range of species.

1.5.1 Influence of body mass/size

Female body mass and size have been shown to strongly predict female competitive ability and performance of competitive behaviours in a range of species, such as African elephants (*Loxodonta africana*) (Archie *et al.*, 2006), feral ponies (*Equus caballus*) (Rutberg & Greenberg, 1990), dwarf mongooses (*Helogale parvula*) (Creel, 2001; Creel & Waser, 1994) and meerkats (*Suricata suricatta*) (Clutton-Brock *et al.*, 2001a; Hodge *et al.*, 2008). In some mammalian species, dominant females also gain weight following a successful take-over, which is likely to be the result of gaining access to higher quality or more substantial food resources (Clutton-Brock *et al.*, 2006). This is particularly important if food is a limited resource (Golabek *et al.*, 2012; Meunier & Kölliker, 2012). For example, when food rations were restricted under experimental conditions, Huck *et al* (1988a) found that dominant female golden hamsters (*Mesocricetus auratus*) accumulated significantly larger food hoards than subordinate females, which consequently influenced reproductive output as subordinate females gave birth to fewer offspring. Subordination usually results in weight loss or slowed weight gain, which may serve to reduce aggression from dominant individuals; in many species larger subordinates are more likely to compete for dominance position, and more importantly are more likely to be successful (Clutton-Brock *et al.*, 2006; Hodge *et al.*, 2008). In a small number of species, there is evidence that body morphology can also change on acquisition of alpha status. Russell *et al*, (2004) found that newly dominant female meerkats gained weight rapidly and developed wider skulls compared to subordinate females, independent of age. Naked mole rats (*Heterocephalus glaber*) also show an increase in body size on acquisition of alpha rank, achieved by elongation of the lumbar vertebrae (O'Riain *et al.*, 2000). Increases in morphology could be under hormonal control, as oestrogen and progesterone have the greatest impact on bone growth (O'Riain *et al.*, 2000).

Female body mass and body length has also been shown to provide reproductive advantages in terms of litter size at birth and weaning weight, with larger and heavier females more likely to produce larger and heavier litters in meerkats (Russell *et al.*, 2004). Reproductive success is higher for larger female dung beetles (*Onthophagus sagittarius*), particularly if they also have larger horns (Watson & Simmons, 2010).

1.5.2 Influence of age

Age is a common covariate of social rank, and has strong positive associations with body mass and experience (Creel, 2001). Age related hierarchies have been reported in chimpanzees (*Pan troglodytes*) (Pusey *et al.*, 1997), mountain goats (*Oreamnos americanus*) (Cote, 2000) and captive bottlenosed dolphins (*Tursiops truncatus*) (Samuels & Gifford, 1997). In communally breeding house mice (*Mus musculus domesticus*), Rusu *et al* (2004) showed that older sisters were more likely to be competitively superior and spatially exclude younger siblings from nest sites, independent of weight asymmetries. However, as females reach relatively old age, age-influenced dominance rank can become unstable in some species; for example once bighorn sheep ewes reach asymptotic mass at approximately seven years of age, age related hierarchies are less fixed as heavier subordinate individuals become more likely to challenge older, lighter dominant ewes (Favre *et al.*, 2008).

1.5.3 Weaponry and ornamentation

Due to general consensus that males are typically more competitive (and the limited number of studies on female traits), female competition is thought to lack the potential required for secondary sexual trait evolution (Watson & Simmons, 2010). In recent years however, there has been increasing interest in sexually selected traits in females (Rubenstein & Lovette, 2009), such as ornamentation (Amundsen, 2000) or weaponry (see Tobias *et al.*, 2012); although there is much debate about whether the evolution of such traits constitutes sexual selection (Ah-King, 2011; Clutton-Brock, 2009b; Gowaty, 2011; Robinson, 2011; Rosvall, 2011a; Rosvall, 2011b; Stockley & Bro-Jørgensen, 2011). Elaborate ornamentation in males is thought to be important for attracting female partners (Clutton-Brock *et al.*, 2006). In some bird species, females may be brighter or more highly ornamented than males which may serve to attract mates, although this trait is also thought to have evolved due to sex differences in territorial defence (e.g. Heinsohn *et al.*, 2005).

Studies of female caribou (*Rangifer tarandus*) (Barrette & Vandal, 1986), antelope species (*Bovidae sp*.) (Packer, 1983) and other ruminants (Roberts, 1996) have suggested that the role of female horns and antlers could be related to intra-sexual competition for limited resources as well as for use in defence against predators. For example, in Soay sheep (*Ovis aries*), females with horns are more likely to initiate and win contests with other individuals during the lambing period, compared to unhorned females (Robinson & Kruuk, 2007). Invertebrate species such as the Onthophagine dung beetle (*Onthophagus sagittarius*) also express horns, and although there are some morphological differences between the sexes, it is possible that horns are used in defence of nesting resources between females (Otronen, 1988). In these beetles, males sometimes help the female to prepare an underground chamber and form a brood ball in which she will lay her eggs (Watson & Simmons, 2010). As the size of the brood ball determines offspring body size, there is potential for female competition over access to dung and male care (Watson & Simmons, 2010), which would potentially increase selection for horn expression. The selective force for female weaponry or ornamentation is however likely to be inhibited by the cost of reproduction and offspring care in females. Selection may therefore favour competitive traits such as increased body mass over investment in elaborate weaponry, due to the relationship between body mass and fecundity.

1.5.4 Personality and dominance

Personality traits describe the behavioural tendencies of individuals that are relatively consistent over time (Bergmüller, 2010; Biro & Stamps, 2008), although behaviour can be altered through learning, physical change or experience (Bergmüller, 2010; Stamps & Groothuis, 2009). Certain personality traits are likely to contribute to individual differences between group members in terms of reproductive success and survival, and the extent and range of competitive behaviour performed (Biro & Stamps, 2008). Activity levels and boldness tend to be positively related to growth or fecundity, but negatively related to survival in the presence of predators (Bergmüller, 2010); for example in damselflies (*Coenagrion hastulatum*) (Brodin & Johansson, 2004), rainbow trout (*Oncorhynchus mykiss*) (Biro *et al.*, 2004) and beef cattle (*Bos taurus*) (Müller & von Keyserlingk, 2006). In an experiment with barnacle geese (*Branta leucopsis*), Kurvers *et al* (2009) found that bolder individuals were more likely to lead the group to food patches and therefore arrive before other group members. However there was no correlation of leadership potential

with social status; leaders had an increased risk of predation and therefore dominant individuals were less willing to lead than lower ranking group members (Kurvers *et al.*, 2009).

Competition for limited resources can be costly in terms of energy, time, risk of injury or death (Briffa & Elwood, 2010). Individuals therefore need to make assessments of their own competitive ability and that of their opponent; referred to as resource-holding potential (RHP) in game theory (Arnott & Elwood, 2009; Parker, 1974). Dynamics of competitive relationships can therefore vary between pairs of animals according to the behaviour and competitive traits of each individual (Drummond, 2006). When one individual is habitually aggressive and the other seldom, then the relationship is described as aggressive-submissive, whereas in aggressive-avoidance relationships the subordinate individual learns to avoid the dominant individual and submissive behaviour is not necessary (Drummond, 2006). However when both individuals are aggressive and reluctant to adopt a subordinate role, repeated violent encounters are likely which could impact on survival, health and reproductive success (Cant & Johnstone, 2000; Drummond, 2006). In highly social groups, females may therefore develop aggressive-submissive relationships as this may improve the chances of successful reproduction. In larger groups, subordinate females may avoid contact with the dominant individual(s) or display signals of submission when in close proximity to them (e.g. grooming behaviour Kutsukake & Clutton-Brock, 2006; Silk, 1982). Non-aggressive traits such as boldness or appeasement behaviours may therefore be important signals of competitive potential.

1.6 Physiological responses to competition within social groups

Social stress can occur when groups are unstable or when new individuals are encountered, resulting in activation of the sympathetic nervous system, which is related to the 'fight or flight' response commonly referred to in behavioural literature (Bartolomucci *et al.*, 2004; Sapolsky, 2002). This activates the hypothalamo-pituitary-adrenal axis (HPA) and stimulates the adrenal glands which are located above the kidneys (Sapolsky, 2002). The adrenal glands have two parts: the adrenal medulla is situated at the core of the gland and produces adrenaline; surrounding this is the adrenal cortex which is responsible for secretion of mineralcorticoids, sex steroids (such as testosterone, progesterone and oestrogen) and glucocorticoids (cortisol/corticosterone) (Sapolsky, 2002).

1.6.1 Glucocorticoids

Glucocorticoids are released in response to both predictable and unpredictable events (Rubenstein, 2007), regulating many of the physiological processes by decreasing metabolism of glucose, increasing metabolism of proteins and fats (to provide energy for muscle use), and temporarily deactivating the immune system to conserve energy (Sapolsky, 2002). In response to activation of the HPA and secretion of glucocorticoids, the adrenal glands become enlarged; evidence of which has been found in sexually mature female voles (*Microtus pennsylvanicus*) in response to group size (Christian & Davis, 1966) and in both male and female Norway rats following social defeat (*Rattus norvegicus*) (Haller *et al.*, 1999). Adrenal gland weight was also shown to positively correlate with population size in rodent species such as meadow voles (*Microtus pennsylvanicus*), Norway rats (*Rattus norvegicus*) and white-footed mice (*Peromyscus leucopus*) (Christian, 1971; Christian, 1975), suggesting that increasing group size results in increased stress, which could be due to competition. Adipose tissue loss has been shown to occur as a result of chronic stress in dominant rodents, with further weight loss from lean tissue observed in subordinate individuals (Tamashiro *et al.*, 2005).

There is mixed evidence regarding the role of social status and glucocorticoid secretion in mammals, with some reports suggesting that dominant individuals secrete lower levels of corticosterone or cortisol than subordinate individuals (Tamashiro *et al.*, 2005), while other reports suggest that subordinates have lower or similar output to dominant individuals (Ely & Henry, 1978; Gust *et al.*, 1996; McGuire *et al.*, 1986; Sapolsky *et al.*, 1983; Stavisky *et al.*, 2001). If dominant individuals acquire and maintain their rank through low-level aggression and threats to subordinates, then a rank related pattern of elevated glucocorticoids in subordinate females could be expected, particularly in some cooperatively breeding species where subordinates could be temporarily evicted from the social group (Creel, 2001; Young *et al.*, 2006). However when social dominance is unstable and dominant individuals are often challenged by subordinates, then the opposite pattern could occur with dominant individuals producing higher levels of glucocorticoids (Creel, 2001; Goymann & Hofer, 2010). Group size has been shown to influence glucocorticoid levels in ring-tailed lemurs (*Lemur catta*), with females in very small or very large groups experiencing higher stress levels, and as a consequence suffering a

reduced probability of successful births and reduced infant survival rates (Takahata *et al.*, 2006).

1.6.2 The effects of sex steroids on female behaviour and physiology

The relationship between social status and androgens (testosterone in particular) has predominantly been investigated in studies of male competition. However female rodent species have been shown to produce testosterone in the adrenal glands, ovaries and placentae, increasing the use of androgen measurements in studies of female behaviour and physiology (e.g. Zielinski & Vandenbergh, 1991). Testosterone is not thought to initiate aggressive behaviour, but instead sustains the behavioural response to competition over a period of time (Bergmüller, 2010; Wingfield *et al.*, 1987). Its release can be increased in anticipation of competition, preparing the individual to engage in aggressive interaction when it is necessary (see Gleason *et al.*, 2009).

Following successful male-male competitive interaction, testosterone levels have been shown to increase in species such as California mice (*Peromyscus californicus*) (Oyegbile & Marler, 2005). This response promotes the 'winner effect', which consequently increases the chances of success in future competitive situations (Oyegbile & Marler, 2005). An influx of testosterone can be rewarding for competitive winners, resulting in conditioned place preference for locations that have been previously successful for female Syrian hamsters (*Mesocricetus auratus*) (Meisel & Joppa, 1994) and a strain of laboratory mice (Martínez *et al.*, 1995). However, high levels of circulating androgens can have inhibitory effects on oestrus cycling and ovulation in other mammal species. Polycystic ovaries in human females produce high levels of circulating androgens (namely testosterone), sometimes resulting in infertility (Franks, 1995). In dogs and primate species, androgen provision delays the onset of puberty (Beach *et al.*, 1983; Goy & Resko, 1972).

There is increasing evidence that testosterone can play a role in female social behaviour in some rodent species such as bank voles (*Clethrionomys glareolus*) (Kapusta, 1998) and laboratory strains of mice (Bronson, 1996), although these tests involved experimental manipulation of hormone levels and therefore may not represent levels that circulate under more natural conditions. In an experimental study with guinea pigs (*Cavia porcellus*), daughters reared in unstable social environments were more likely to show masculinised

behaviour, such as aggressive play fighting and male-typical courtship patterns (Kaiser & Sachser, 2005). The presence of androgens can also affect maternal behaviour by impairing pup retrieval in rats (Bridges *et al.*, 1973) or maternal nest building in rabbits (Anderson *et al.*, 1970).

There is strong evidence to suggest that androgen exposure in-utero could provide a distinct advantage in competitive environments. Androstenedione (A4) is secreted in the ovaries and transformed to testosterone in the placenta, passing to foetuses of both sexes (Drea, 2009). Pre natal exposure to testosterone is thought to be linked to longer anogenital distance in female mice (Palanza *et al.*, 2005), particularly if females are positioned between two male siblings in-utero (Vom Saal, 1989). In a study on a laboratory strain of mice, female neonates that were situated between two brothers in-utero were shown to have longer oestrus cycles, delayed sexual maturity and were more aggressive towards other females at sexual maturity (Vom Saal, 1978). Androgen exposure has therefore been suggested as a rank-related maternal effect, providing competitive benefits for offspring born to dominant females (Dloniak *et al.*, 2006). For example, in a study by Clutton-Brock *et al* (2006) dominant female meerkats had higher levels of circulating testosterone during pregnancy compared to subordinate females. However, high levels of androgens may also affect ovarian activity and subsequent fertility (Glickman *et al.*, 1993; Glickman *et al.*, 1998; Koren & Geffen, 2009; Packer *et al.*, 1995; Rutkowska *et al.*, 2005; Walters *et al.*, 2008). High ranking female baboons can sometimes have difficulty in completing a pregnancy or suffer a delay in the onset of reproduction, which may be a consequence of high levels of circulating androgens (Packer *et al.*, 1995). Spotted hyenas (*Crocuta crocuta*) have been extensively studied due to female biased dominance hierarchies and the degree of masculinisation exhibited by females (Frank, 1986). Adult females are larger and more aggressive than males, gaining priority access to food which subsequently assures an adequate supply of food for their offspring (Tilson & Hamilton, 1984). Pre natal androgen secretion influences adult body weight, aggressive behaviour and masculinisation of genitalia (Neaves *et al.*, 1980). In a study of rock hyraxes (*Procavia capensis*), Koren *et al* (2006) found that females had similar testosterone output to males and were behaviourally dominant over them, however lower ranking females had even higher levels of testosterone than higher ranking females. The authors suggested that high ranking females were better equipped to cope with high levels of androgens, perhaps through down-regulation of receptors and enzymes, which enabled them to deal with the potential negative

consequences of androgen production (Koren & Geffen, 2009). Therefore female quality may influence how effective individuals are in negating potential costs of circulating androgens.

1.7 Cooperative breeding systems

Cooperative breeding is a term used to describe a social group in which members assist with rearing young other than their own offspring (Sayler & Salmon, 1971). Helpers may be sub-adults, other breeding group members or non-breeding adults, performing a number of duties. Direct offspring care may consist of food provisioning and grooming, while indirect care duties include burrow defence or group foraging. Cooperative behaviours also help to maintain group cohesion (Hayes, 2000). Understanding the factors that influence the evolution of cooperative breeding is a subject of great interest within evolutionary biology. Numerous hypotheses have been proposed to explain the occurrence of cooperative breeding, with many studies focusing on birds due to a wide range of species exhibiting this behaviour (Arnold & Owens, 1998; Cockburn, 1998; Dickinson & Hatchwell, 2004; Iwaniuk & Arnold, 2004; Jetz & Rubenstein, 2011). Relatively fewer mammals have been reported to exhibit cooperative breeding, but species examples are widely distributed across taxa including canids, primates and rodents (Solomon & French, 1997).

Kin selection theory has played a pivotal role in explaining social behaviour in many animal species, suggesting that individuals should behave more altruistically towards their relatives as they share more alleles that are identical by descent (Hamilton, 1963). Copies of these genes are then more likely to be passed on to the next generation, increasing the opportunities for altruism as relatedness between the group extends (Hamilton, 1964a; Hamilton, 1964b). Kin selection theory suggests that competition between relatives may be maladaptive, as the benefits of helping kin to rear their offspring or defend them against intruders outweighs the costs associated with competing (Clutton-Brock, 2002; Rubenstein, 2012). However the potential for competition between relatives exists, and on occurring, reduces the kin-selected benefits of altruism (West *et al.*, 2002). Studies of competition between relatives are rare, but there are examples in dwarf mongooses (*Helogale parvula*) (Creel & Creel, 1991), meerkats (*Suricata suricatta*) (Clutton-Brock *et al.*, 1999), striped mice (*Rhabdomys pumillo*) (Schradin *et al.*, 2010) and red fronted lemurs (*Eulemur rufifrons*) (Kappeler & Fichtel, 2012). With increasing group size, red

fronted lemurs need to compete for reproductive opportunities with their close relatives, resulting in reduced chances of successful reproduction and high risks of eviction from the group during the breeding season, even in the absence of clear social ranks (Kappeler & Fichtel, 2012). In the wild house mouse, females predominantly interact with familiar sisters and unfamiliar, unrelated individuals (Rusu & Krackow, 2004). Familiarity with an individual may however reduce the amount of aggressive behaviour observed on first meeting (Latham & Mason, 2004). Recently, Weidt *et al* (2008) showed that unrelated female house mice had higher reproductive output if they nested with a female they previously shown high association with, suggesting that female characteristics may be important in predicting reproductive success in communally breeding species.

As there are numerous differences between mammalian and avian species in terms of physiology, offspring development and provisioning behaviours, there is much interest in the evolution of cooperative breeding. Traditionally the focus has been to investigate the benefits of cooperative breeding and as many cooperative social groups consist of close relatives, kin selection theory has been used as an ultimate explanation for the occurrence of cooperative breeding (Bergmuller *et al.*, 2007; Clutton-Brock, 2002; Komdeur, 2010). Despite strong evidence for inclusive fitness benefits in some species, there are also examples of unrelated groups cooperatively rearing offspring, such as in some *Polistes* paper wasp species (Field & Cant, 2007). Unrelated helpers may be forced to work harder as payment for reproducing but this may also increase their chances of inheriting the breeding position in the future (Kokko *et al.*, 2002). However Lukas and Clutton-Brock (2012a) recently suggested that reproductive suppression may be necessary for the maintenance and success of cooperative breeding systems when unrelated helpers are present. A change in reproductive skew would therefore be potentially detrimental to the level of cooperation between individuals, resulting in instability which could threaten the survival of the group/colony.

Cooperative breeding can be divided into two categories, depending on the degree of reproductive skew observed within social groups: these are singular and plural breeding systems.

i) Singular breeding systems (or cooperative breeding)

The most common cooperative breeding system in mammals consists of social groups with just one female dominating reproduction, producing offspring that typically remain in the

natal nest and are often reproductively suppressed as adults (Sayler & Salmon, 1971). This is commonly referred to as cooperative breeding. Most non-breeding individuals assist directly or indirectly to care for the offspring of the breeding female and therefore there could be high levels of conflict between group members. The pay-to-stay hypothesis has been suggested to explain why subordinate group members remain in their natal nest site, rather than disperse to breed, as the benefits associated with staying should outweigh the costs of dispersal and joining another group (Bergmuller *et al.*, 2007). More recently (Lukas & Clutton-Brock, 2012a) have shown that the evolution of cooperative breeding was restricted to monogamous lineages in mammals. As a result of this cooperative group members should have a high coefficient of relatedness and therefore non-breeding helpers gain inclusive fitness benefits from helping to rear offspring (Bergmuller *et al.*, 2007). Species examples of singular breeders include pine voles *Microtus pinetorum* (Fitzgerald & Madison, 1983), alpine marmots *Marmota marmot* (Blumstein & Armitage, 1999) and naked mole rats *Heterocephalus glaber* (Jarvis, 1981).

ii) Plural breeding systems (or communal systems)

Plural breeding systems describe social groups with one or more breeding female rearing their combined young in a single nest site and all females assist with offspring care (Sayler & Salmon, 1971). This is commonly referred to as communal breeding. It is the most egalitarian of cooperative systems as there is low reproductive skew, however this system is relatively rare in both mammalian and avian species (Gilchrist, 2007). Communal breeding systems are thought to have evolved from plural breeding ancestors with polygynous mating systems (Lukas & Clutton-Brock, 2012a). As most sexually mature females tend to breed in this system, the potential for competition between females is expected to be relatively lower compared to cooperatively breeding groups (Gilchrist, 2007). Examples of communally breeding species include wild house mice (*Mus musculus domesticus*) (Konig, 1997; Manning *et al.*, 1995), banded mongooses (*Mungos mungo*) (Gilchrist, 2006), and spotted hyenas (*Crocuta crocuta*) (Frank, 1986). Some species, such as the wild house mouse, also communally nurse the offspring born into a shared nest which further increases potential lactational demands on breeding females (Konig, 2006) (for more detail on communal nursing see Section 1.7.3 below).

1.7.1 Benefits of cooperative/communal breeding

Thermoregulatory benefits have previously been used to explain the occurrence of communal nesting, particularly as many cooperative species give birth to altricial young (Roulin, 2002). Temperature and precipitation levels may therefore be an important factor in the evolution of cooperative breeding systems; the importance of such factors have previously been discussed in comparative studies (e.g. Hatchwell & Komdeur, 2000). Altricial species can only maintain body temperature through huddling with group members to reduce surface-to-volume ratio which reduces energy spent on thermoregulation (Edelman & Koprowski, 2007). Therefore as ambient temperatures reduce, endothermic species should communally nest to reduce energy expenditure of maintaining body temperature, as described in a study of Abert's squirrels (*Sciurus aberti*) (Edelman & Koprowski, 2007). Offspring are therefore more likely to have increased growth rates as a result of better nutrition and thermoregulation (Sayler & Salmon, 1969). The increase in group size also increases the number of vigilant and protecting adults, further increasing the chances of pup survival (Hayes, 2000; Sayler & Salmon, 1971).

Cooperative breeding is thought to have evolved in lineages with socially monogamous mating systems (Lukas & Clutton-Brock, 2012a) as this leads to high levels of average kinship between group members. The degree of relatedness between group members is therefore influential on litter size and weight in many cooperative and communally breeding species (Konig, 1994a), and is thought to result in increased reproductive output compared to solitary rearing (Konig, 1993). For example, young female dormice (*Glis glis*) gave birth earlier if they communally nested with a sister, compared to nesting alone, providing their offspring with more time to develop and grow before the hibernation period commenced (Pilastro *et al.*, 1996). Synchronisation of births in communally breeding species is thought to result in a number of benefits, such as dilution effects to reduce the risk of predation, increased efficiency of parental care by pooling offspring and sharing care duties, and it also minimises the risk of infanticidal behaviour (Poikonen *et al.*, 2008).

1.7.2 Costs of cooperative/communal breeding

Most cooperative and communal species give birth to altricial offspring that require relatively high levels of parental care; a cost which is shared between mothers and/or helpers (Lukas & Clutton-Brock, 2012b). The costs associated with parental care therefore

increases when the number of dependent litters present in the nest increases. Social group size has previously been shown to negatively influence reproductive success, for example there is reduced litter survival when the number of female group members increases within meerkat populations (Hodge *et al.*, 2008). There are a number of constraints which may affect reproductive success in mammals, including availability of food, shelter, protection and assistance with care (Stockley & Bro-Jørgensen, 2011). In competitive environments such those found in cooperative breeding systems, dominant individuals are more likely to gain priority access to limited resources, favoured feeding areas and/or reproductive opportunities compared to other group members (Clutton-Brock *et al.*, 2008). Offspring survival may also be reduced when resources are limited, with females reportedly performing infanticide to reduce the energetic burden related to offspring care (Weber & Olsson, 2008). Within cooperatively caring species, the majority of non-reproducing females remain reproductively suppressed throughout their life and therefore gain fitness benefits by caring for related offspring (Field & Cant, 2007; Komdeur, 2010; Wright *et al.*, 2009). Dominant individuals however could gain benefits from minimising conflict and enabling relatives to occasionally breed, particularly in conditions where dispersal is possible (Cant & Johnstone, 2000), as this may avoid the risk of losing helpers and reduce the risk of take-over attempts by subordinate females (Creel & Waser, 1994). For example, subordinate meerkats have been observed to test the fighting ability of the dominant female, particularly within small groups (Cant & Johnstone, 2000).

Reproductive suppression occurs when one or more dominant individuals impose endocrine or behavioural controls on the breeding of more subordinate individuals. As costs associated with dispersal are often high, there is usually no better option than for the subordinate individual to help (Cant & Johnstone, 2009; Mock & Parker, 1997). Suppression can occur directly through harassment and aggression, or indirectly through signals of dominance status (Creel *et al.*, 1992; Wasser & Barash, 1983). This results in disruption of the oestrus cycle, failed implantation (Ma *et al.*, 1998), delayed sexual maturity (Drickamer, 1974) or spontaneous abortion (Clutton-Brock *et al.*, 2008). In a cooperatively breeding species, pregnant subordinate meerkats (*Suricata suricatta*) are generally evicted from the group by high ranking females, leading to a chronic stress response which increases the risk of spontaneous abortion (Young *et al.*, 2006). Increasing group size can also result in reproductive suppression in some communally breeding rodent species through hormonal control (Ma *et al.*, 1998; Van Der Lee & Boot, 1955; Van Der

Lee & Boot, 1956). By delaying the conception of competitive social partners/group members, females reduce the potential for future feeding competition and also the number of offspring requiring care (Wasser & Barash, 1983).

Infanticidal behaviour (i.e. the killing and sometimes consumption of dependant young) is the most extreme form of reproductive control. It may be performed by males or females, and not necessarily by unfamiliar individuals, as is often reported (Agrell *et al.*, 1998; Clutton-Brock *et al.*, 1998; Hrdy, 1979; Poikonen *et al.*, 2008; Sherman, 1982; Townsend *et al.*, 2007; Tuomi *et al.*, 1997). A recent study on banded mongooses (*Mungos mungo*) found that females were more likely to suffer an increased risk of infanticide against their offspring if they gave birth before other group females (Hodge *et al.*, 2011); a similar pattern was also found in common marmosets (*Callithrix jacchus*) (Saltzman *et al.*, 2008). Infanticidal females are more likely to be of higher rank and unrelated to the affected female due to the costs of killing related young (Nicolson, 1987). Nevertheless, there should be strong selection for birth synchrony within communally breeding groups as this has been shown to reduce the rate of infanticidal behaviour in many species (Ebensperger, 1998; Poikonen *et al.*, 2008). Synchronised births potentially make discrimination between combined offspring difficult and therefore individuals risk losing their own offspring if they perform infanticidal behaviour (Ebensperger, 1998; Maestripieri & Alleva, 1991; Poikonen *et al.*, 2008).

1.7.3 Costs and benefits of shared parental care in cooperative breeding systems

Perhaps the most costly maternal behaviour performed by some communally nesting species is communal nursing, where females share milk between their own young and those of other mothers within their group (Hayes, 2000; Konig, 2006). Non-offspring nursing has been reported in almost all major taxonomic groups of mammals (Packer *et al.*, 1995) and may bring immunological benefits to offspring as other lactating females are likely to have encountered a range of pathogens (Konig, 2006). Communal nursing increases the amount of time females have to gather food and provides rest periods to replenish energy for lactation (Hayes, 2000). Under this condition, the potential for conflict is significantly higher due to the high energetic demands on the lactating females (Konig, 2006; Maestripieri & Alleva, 1991), although the costs associated with non-offspring nursing are reduced if females feed closely related young (Manning *et al.*, 1995; Packer *et*

al., 1995). If reciprocated, non-offspring nursing can also provide benefits to young. For example the interval between nursing bouts can be shortened and offspring may receive a more constant and plentiful supply of milk from multiple lactating females (Lewis & Pusey, 1997). Mothers also benefit from reciprocal nursing behaviour as it provides them with more time to forage when another lactating female is nursing and protecting their young (Lewis & Pusey, 1997). Female mammals generally lose weight during the lactation period, even when solely feeding their own offspring, and need to lay down fat reserves before the breeding season in order to cope. They may also undergo physiological changes in the liver, kidneys and digestive tract (Speakman, 2008). By reproducing in cooperative conditions, females may nurse more offspring than they have given birth to, which may exceed their energetic capacity (Konig, 1993). In addition, non-offspring nursing can have negative physiological effects on an individual level, for example if females do not equally care for young then demand may be increased for the female providing more milk (Konig *et al.*, 1988).

Although shared parental care has been described as one of the major benefits of cooperative and communal breeding, the potential for competition between group members is increased and dominant individuals may enforce helpers to care for their offspring (Clutton-Brock & Parker, 1995; Vehrencamp, 1983). In the superb fairy wren (*Malurus cyaneus*), helpers that are experimentally removed when dependent offspring are present are attacked by the dominant male on their return; conversely, if helpers are removed during the non-breeding season they are not attacked on their return (Mulder & Langmore, 1993). Meerkat males behave aggressively towards subordinates that fail to provide food for pups (Clutton-Brock *et al.*, 2005), but lazy individuals are not thought to be evicted from the group (Clutton-Brock, 2002). Subordinates should therefore adopt compensatory behaviours by helping to care for the offspring of dominant females, as this could reduce the amount of aggressive behaviour they receive (Bergmuller & Taborsky, 2005). Larger helpers in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*) however have increased survival prospects if they are expelled from the social group compared to smaller helpers and therefore they may be less willing to pay to stay (Bergmuller & Taborsky, 2005). Punishment and harassment are likely to be important in the maintenance of cooperative behaviours, even if it is immediately costly for the punisher due to increased risk of injury (Jensen, 2010); however, threats are more likely than punishment in cooperatively breeding species (Cant, 2011; Cant & Johnstone, 2009; Jensen, 2010).

Mammalian offspring are entirely dependent on milk throughout the first stage of lactation and therefore nutrients essential to growth are gained from the milk of lactating females (Langer, 2008). As there are likely to be two or more reproducing females present in communal systems, offspring could potentially suckle from females other than their own mother, between which milk quality may vary (Hinde & Milligan, 2011; Langer, 2008). Milk quality can be affected by diet, frequency of nursing, age and weight of females (Landete-Castillejos *et al.*, 2005). Litter size in-utero can also determine mammary growth in pregnant females (Jameson Jr, 1998). Mothers have an upper limit on successful feeding and weaning of young and reach a physiological maximum (Konig *et al.*, 1988; Rogowitz, 1996; Sikes & Ylonen, 1998), therefore female milk quality may be reduced when caring for large litters or if lactation is performed for longer periods than anticipated (Fuchs, 1982; Knight *et al.*, 1986; Manning *et al.*, 1995). The main energy source of milk generally consists of fats, which under standard conditions, increases in quantity during peak lactation (Konig, 2006). Under conditions of high demand however, the proportion of milk solids can be reduced and therefore the energetic content being provided to offspring is subsequently reduced (Rogowitz, 1996). Females are unable to increase the quality of their milk proportionally over the lactation period. As a consequence larger litters grow more slowly and have a lower overall weaning weight (Konig *et al.*, 1988). If one or more females in the communal nest reduced the amount of care they provided, this could have serious negative implications for their own offspring, particularly if the social partner had lower quality milk (Gerlach & Bartmann, 2002; Hinde & Milligan, 2011; Langer, 2008).

1.7.4 Offspring competition & development in the cooperative and communal nest

Sibling competition has been extensively studied in avian species as unpredictable provisioning of young is associated with nesting aggression and contest competition (Drummond, 2001). Sibling competition can range from extreme aggression (such as siblicide) to scramble competition, where overt aggression is highly unlikely (Bautista *et al.*, 2005; Drummond, 2006). Evidence of obligate siblicide (i.e. competition that generally results in the death of a sibling) is incredibly rare in mammal species, although facultative siblicide (i.e. fighting between siblings generally without causing death) can occur if mothers are unable to maintain milk supply (Hofer & East, 2008; Trillmich & Wolf, 2008). Unlike birds, many mammals adopt immobile nursing postures over their offspring and

there is little opportunity for mothers to be selective over the offspring they nurse (Hudson & Trillmich, 2008). This results in scramble competition between the dependent young and individuals that are larger at birth tend to have advantages over littermates in competition for milk (Mock & Parker, 1997). Sibling competition has also been shown to be an important factor in pre natal growth rate in mammalian species (Stockley & Parker, 2002), which could influence post-natal competitiveness.

In communal nests, birth order is therefore important for sibling competition; by giving birth first, offspring gain size and weight advantages over the subsequent litters, enabling them to effectively compete for access to lactating females (Hodge *et al.*, 2009; Mock & Parker, 1997). Older siblings are therefore more likely to benefit from size-related advantages in scramble competition with other littermates, and this may be emphasised when younger siblings are also the weaker sex (Mock & Parker, 1997). In an experiment with twin hyena litters, Benhaiem *et al* (2012) found that dominant sisters were more successful against subordinate brothers than vice versa, resulting in growth benefits. In species where males tend to be the dominant sex (e.g. barn swallows *Hirundo rustica*), males are likely to outcompete their sisters via begging behaviour (Bonisoli-Alquati *et al.*, 2011). In birds, younger chicks try to compensate for their size disadvantages by begging more intensely (Roulin *et al.*, 2009). However under some circumstances it is better for subordinates to adjust their submissiveness as the risk of starvation increases, as this has been suggested to improve chances of receiving food (Benhaiem *et al.*, 2012). Begging behaviour by offspring is common in many avian species such as the barn swallow (Bonisoli-Alquati *et al.*, 2011), in insect species such as the common earwig (*Forficula auriculari*) (Mas *et al.*, 2009) and in mammalian species such as meerkats (*Suricata suricatta*) (Manser *et al.*, 2008). Both avian parents and mammalian helpers can provision young according to honest signalling of quality by begging offspring (Godfray, 1991), however mammalian mothers cannot regulate the flow of milk to individual pups (Hudson & Trillmich, 2008).

In most mammal species the number of teats generally outnumbers litter size (Gilbert, 1986). However if the number of dependent offspring exceeds the number of teats, as may occur in communal nest environments, higher levels of sibling competition could be expected. Access to nipples may also be restricted as a result of the mother's resting position (e.g. Fraser *et al.*, 1995). As offspring tend to remain attached to a nipple until it is depleted, unsuccessful offspring may need to wait for another nursing opportunity before

they can feed (Cramer & Blass, 1983), which may have long-term impacts on growth and survival. In larger litters, female house mice (*Mus musculus domesticus*) increase the total amount of milk they produce, but each pup receives less milk and has a lower weaning weight than offspring born in a smaller litter (Konig, 1997). Certain teats may also be more productive and therefore priority access to those teats could further increase competition between offspring (Mock & Parker, 1997). In the domestic pig (*Sus scrofa*), anterior nipples are the most productive and offspring grow more quickly if they gain priority access to those nipples (Fraser, 1990). In felids, binturongs and common opossums, posterior teats are more productive (Mock & Parker, 1997). Once again, heavier and older offspring may have a competitive advantage due to improved motor ability, enabling them to reach teats more rapidly than lighter offspring (Bautista *et al.*, 2005).

As discussed above, offspring growth is associated with relative competitiveness of offspring, potentially resulting in long term benefits for those individuals who can outcompete their littermates (Royle *et al.*, 1999). Offspring with greater body mass also have a smaller body volume to surface ratio, resulting in reduced heat loss compared to smaller offspring (Rodel *et al.*, 2008). However, larger individuals have higher energy requirements and are therefore more vulnerable to food shortage (Uller, 2006). Other long-term consequences for offspring born into communal or cooperative nests include delayed dispersal, with some individuals remaining in the natal nest for life, and/or also delayed age of first reproduction (Bergmuller *et al.*, 2007; Hatchwell & Komdeur, 2000). In an experimental study on guinea pigs (*Cavia aperea porcellus*), cortisol levels were found to be increased in larger litters during early stages of development when competition was increased (Fey & Trillmich, 2008), which may have long-term consequences; glucocorticoids help to increase vigilance which may enable hungry offspring to respond quickly to parent or helper presence (Roulin, 2001), however chronic stress can impair immune and reproductive function (Munck *et al.*, 1984). Therefore long-term fitness could be determined by how well adapted offspring are to potential future situations.

1.7.5 Maternal effects in the cooperative nest

The success of individuals depends not only on the ability to survive and successfully reproduce, but to produce offspring that are more successful than the offspring of competitors (Cunningham & Birkhead, 1998; Dawkins, 1989). This may be achieved by mating with high-quality individuals or through differential investment in reproduction

(Cunningham, 2003; Cunningham & Russell, 2001a). Maternal effects describe the condition when a mother's phenotype or environment can influence the phenotype of offspring, over the direct effect of transmitted genes (Marshall & Uller, 2007). There are several phases in which maternal effects can be implemented: firstly during the pre-reproduction stage via effects of mate choice or timing of reproduction; secondly during early reproduction via adjustments to litter (or clutch) size, mass, gender or quality of offspring (Cunningham & Russell, 2001a; Cunningham & Russell, 2001b; Gil, 2003; Groothuis *et al.*, 2005; Russell & Lummaa, 2009); thirdly during the late reproductive phase through differences in offspring care (Hager & Johnstone, 2007); finally during the post-independence stage through interactions with offspring that remain in the natal nest (Russell & Lummaa, 2009).

Maternal effects can be anticipatory, meaning mothers may increase investment in their offspring before birth to provide them with competitive advantages in potentially challenging environments. For example if the environment is perceived to be relatively harsh or opportunities for daughters to reproduce is limited, mothers may adjust investment towards male offspring who could outcompete female littermates and disperse from the natal area at sexual maturity and breed in other territories (Russell & Lummaa, 2009; Tschirren *et al.*, 2012). Alternatively, where mothers may benefit from the number of potential helpers in the nest (e.g. in a communal breeding system), females may increase investment in daughters (Russell & Lummaa, 2009; Silk, 1983; Simpson & Simpson, 1982). Strategies may be adjusted according to female competitiveness; for example, by reducing the number of male offspring produced, females can reduce overall maternal investment, as males are usually larger at birth and require more maternal care (Leimar, 1996; Rivers & Crawford, 1974; Trivers & Willard, 1973). However, female quality could also affect the ability to alter investment, as less competitive females may be not be able to access sufficient or quality resources such as food sites compared to more competitive or higher quality females (Clutton-Brock *et al.*, 2006; Vogel, 2005).

Some species may adopt different rearing strategies according to the environment in which they reside; for example, African striped mice (*Rhabdomys pumilio*) rear young in solitary nests in eastern grasslands and nest communally in arid western areas (Schradin & Pillay, 2005). Solitary females are territorial and vary in competitive ability, resulting in competition between neighbours (Schradin & Pillay, 2005). In an experimental study, Kinahan and Pillay (2008) found that litter size and mass was significantly increased for

dominant females when they were housed adjacent to subordinate females. Subordinate females however increased the amount of time they spent in contact with offspring when unfamiliar dominant females were rearing young close by (Kinahan & Pillay, 2008). This suggests that subordinate females were able to perceive the risks to their offspring and adopted a defensive strategy in response.

Experience of living in an unstable social environment can provide offspring with competitive advantages, particularly in high-density environments (Kaiser & Sachser, 2009). In an experiment with guinea pigs (*Cavia aperea porcellus*), Kaiser *et al* (2003) found that females living in unstable social environments showed behavioural masculinisation, increased testosterone output and adapted androgen receptors. However, their offspring showed delayed development of the adrenocortical system and brain development (Kaiser *et al.*, 2003), suggesting a trade off in terms of social stress. Interestingly, male guinea pigs born in high-density environments can gain advantages from delayed maturation as they were more likely to be tolerated in the presence of a dominant male. In addition, they also had the best chance of gaining alpha status in later life, due to the reduction in directed aggression before sexual maturity (Kaiser & Sachser, 2009).

As both social experience and maternal effects serve to control and modulate the quality and adaptability of offspring, they can also potentially modify intensity and direction of selection, and therefore evolutionary change (Kaiser & Sachser, 2005). As competition between offspring in the communal nest is likely to be increased, there could be selection for increased pre natal growth to provide competitive advantages. However the costs associated with producing larger offspring at birth could lead to selection for shorter gestation length (Stockley & Parker, 2002). Due to these effects on maternal investment, it is therefore possible that maternal effects are common among communally breeding species, as strategies adopted throughout reproductive life can influence both direct and indirect fitness of participating mothers.

1.8 Study species – Wild house mice (*Mus musculus domesticus*)

House mice offer a number of advantages for the study of female competition within a communally breeding system. They exhibit both communal care and communal nursing of young and there are numerous studies reporting effects of relatedness and familiarity of

social partners on reproductive success (Konig, 1993; Konig, 1994a; Konig, 1994b; Konig & Lindholm, 2012; Manning *et al.*, 1995; Sayler & Salmon, 1969; Sayler & Salmon, 1971), as well the occurrence of maternal aggression, reproductive suppression and infanticide (Konig, 1994a; Lidicker, 1976; Maestripieri & Alleva, 1991; McCarthy & Vom Saal, 1985; McCarthy & Vom Saal, 1986; Palanza *et al.*, 2005; Palanza *et al.*, 1996; Rowe & Redfern, 1969; Vom Saal *et al.*, 1995). Wild mice differ greatly from laboratory strains in terms of lifespan, reproduction and growth (Harper, 2008), and also lack the genetic similarities shown between unrelated communal partners in studies of laboratory mice, providing an ideal species to study individual (and potentially inherited) traits, which may be important in competition.

The house mouse (*Mus musculus domesticus*) is a small murid rodent species, distributed across many parts of the world (Bronson, 1979). House mice are nocturnal, but mainly active around dawn and dusk (Mackintosh, 1981), feeding on a variety of food sources including cereals, roots, seeds and insect larvae (Rowe, 1981). House mice live in commensal populations within man-made structures, or can also survive in feral populations, largely independent of man (Berry, 1981; Rowe, 1981). In commensal populations, male house mice are highly territorial, with home ranges less than 10 m^2, whereas in feral populations the home ranges of males can vary, reaching up to 1000 m^2, and therefore territorial defence may not be as rigid (Bronson, 1979). Life expectancy can also vary according to the habitat in which they reside. Mice in commensal populations with restricted predation have a life expectancy of approximately 200 days (Konig & Lindholm, 2012), while feral mice in Russia have been reported to live for over 630 days (Berry & Bronson, 1992). Breeding may be seasonal in feral populations, depending on the climate, but in commensal habitats breeding tends to occur all year round (Pelikan, 1981).

Groups generally consist of a dominant male who holds the territory, with or without subordinate males, several breeding females with their offspring and some non-reproducing females (Hurst & Barnard, 1992; Reimer & Petras, 1967). Subordinate males are non-territorial and their movements are restricted by the dominant male in the territory (Crowcroft & Rowe, 1963). Dominant males actively defend their territories using scent marking behaviour and aggression towards roving males (Hurst, 1987). Females are not confined to a single male territory, with home ranges potentially extending across a number of male territories (Hurst, 1990a). Competition between males is thought to be primarily for access to females (Crowcroft & Rowe, 1963; Fredericson & Birnbaum, 1954;

Rowe & Redfern, 1969; Scott & Fredericson, 1951), as food tends not to be a limiting resource in commensal populations (Konig, 1994b).

1.8.1 Reproduction

House mice are a very adaptable species and rapidly reproduce during their lifetime (Konig & Markl, 1987). The reproductive biology of the house mouse enables high levels of reproduction throughout their lifespan. In some laboratory strains, mice have been shown to reach sexual maturity as early as four weeks of age (Berry & Bronson, 1992), however most wild-derived mice reach maturity at six to eight weeks and breed every four weeks thereafter (Berry, 1981). Females are attracted to dominant males and mate with the territorial male(s) within their home range (Hurst, 1986). Average litter size of wild-derived mice is approximately four to eight (Konig & Markl, 1987; Pelikan, 1981), although first litters tend to be smaller than subsequent litters, and litter size reduces with female age (Konig & Markl, 1987). Females exhibit post-partum oestrus on giving birth and therefore lactation and pregnancy can be simultaneous, increasing lactation length to approximately 28 days after birth (Konig & Markl, 1987; Williams & Scott, 1953). Females can reproduce rapidly, but towards the end of their lifespan they suffer a reduction in fertility due to depletion of oocytes and deterioration of the uterus (Berry & Bronson, 1992).

House mice are communal breeders, with approximately two to three females pooling their young in a single nest, often communally nursing indiscriminately (Manning *et al.*, 1995; Sayler & Salmon, 1971). Due to the low dispersal rate of females, communal nursing tends to occur more frequently between kin (Konig, 1993; Konig, 1994b; Rusu & Krackow, 2004). However if nest sites become crowded and there is competition for reproductive opportunities, females may need to disperse and join an existing group of unfamiliar (and potentially unrelated) females (Sayler & Salmon, 1971). Although communal nesting has been shown to provide reproductive advantages in terms of birth weight, growth rate and litter size compared to solitary nesting (Hayes, 2000; Konig, 1993; Sayler & Salmon, 1971), communal breeding conditions also increase the risk of infanticide and reproductive suppression (Hurst, 1987; Konig, 1994a; Manning *et al.*, 1995; Palanza *et al.*, 2005). Indeed reproductive output has been shown to increase when females were paired with a previously preferred partner (Weidt *et al.*, 2008). Social partner choice therefore has important consequences on reproductive success in wild house mice.

Female wild house mice were previously thought to be non-aggressive except during pup defence, commonly referred to as maternal aggression (Mackintosh, 1981). However, competitive behaviour has been shown to occur in other situations, influencing population dynamics of social groups (Hurst, 1987; Palanza *et al.*, 2005; Palanza *et al.*, 1996). Palanza *et al* (2005) suggest that aggression serves to expel same-sex rivals and that dominance may be unstable if a female cannot expel a competitor. Therefore females may also compete for access to nest sites, particularly in densely populated areas (Konig & Lindholm, 2012). When groups are crowded (i.e. more than three individuals in a nest site), females may become reproductively suppressed (Hurst, 2005). Reproductive success can also be affected by relatedness and familiarity of the social partner that individuals nest with (Konig, 1994a; Palanza *et al.*, 2005). This is thought to be the result of reproductive suppression through aggressive acts (Palanza *et al.*, 2001) or mating interference. In addition, if a female loses her litter shortly after birth it is not known if she would remain at the nest site and care for her nest partner's offspring, or if she would refrain from communal nursing or attempt to disperse (Konig, 1994a).

1.8.2 Scent communication

Olfaction is the dominant method of communication between house mice (Johnson, 1973). The molecular components of male urine provide fixed and variable information about sex (Roberts *et al.*, 2010), identity (Cheetham *et al.*, 2007), social and health status (Hurst & Beynon, 2004) and therefore territory ownership (Hurst, 1993; Hurst & Beynon, 2004). Spatial and temporal distribution of scent marks provide females with information on male territorial defence, as females have been shown to prefer males that maintain an exclusively marked territory (Rich & Hurst, 1998). Dominant males deposit numerous and relatively small and streaky scent marks throughout their territory, while subordinate males void their urine into large pools (Desjardins *et al.*, 1973; Malone *et al.*, 2005). Territorial males attack subordinate males that deposit competitive scent marks in their area, and they quickly counter-mark rival scents in their territory (Hurst, 1990b; Hurst & Beynon, 2004). Following male-male competition, defeated males reduce their rate of scent marking in the presence of a dominant male's marks (Desjardins *et al.*, 1973). This is thought to be an advertisement of subordinate status and enables the individuals to be tolerated in another male's territory (Hurst *et al.*, 2001b). Newly dominant males reduce the size of scent marks and repeatedly deposit them across the same area over several hours (Desjardins *et*

al., 1973; Hurst & Beynon, 2004). Replenishing scent marks maximises freshness, which strongly influences female mate choice (Hurst *et al.*, 2001a).

In competitive environments, competitors usually sniff towards each other from a distance and then flee or attack on the basis of information gained from volatile scents (Hurst, 1993). Involatile components are used by animals to recognise familiar individuals through direct contact with scent marks (Hurst & Beynon, 2004). If volatile signals change due to changes in social status then close investigation with scent marks updates the link to identify the individual (Hurst & Beynon, 2004).

1.8.3 Chemical signals - volatiles

Male mouse urine contains numerous androgen dependent components that mediate aggression between males, namely 2-*sec*-butyl-4,5-dihydrothiazole (thiazole), 2,3-dehydro-*exo*-brevicomin (brevicomin), E,E-α-farnesene and E-β-farnesene (Andreolini *et al.*, 1987; Hurst & Beynon, 2004; Novotny *et al.*, 1990; Novotny & Wiesler, 1999). All of these volatiles act to elicit a sniffing response in females and are produced by all mature male mice (Hurst & Beynon, 2004; Jemiolo *et al.*, 1991), although higher concentrations of thiazole and farnesenes are produced by dominant males (Harvey *et al.*, 1989; Hurst & Beynon, 2004; Jemiolo *et al.*, 1991; Novotny *et al.*, 1990). Farnesenes are produced in the preputial gland, which is situated close to the urethra to enable the waxy volatile compounds to be distributed with other volatile and involatile components in the urine (Harvey *et al.*, 1989; Hurst & Beynon, 2004; Novotny *et al.*, 1990). The preputial gland of dominant males can be twice as large as those of subordinate males (Novotny *et al.*, 1990), increasing the urinary concentration of farnesenes (Harvey *et al.*, 1989), subsequently resulting in high physiological demands (Malone *et al.*, 2005).

1.8.4 Chemical signals – Major Urinary Proteins

Scent marks contain high concentrations of protein, 99% of which are major urinary proteins (MUPs), which have a high affinity for thiazole and brevicomin in male urine (Humphries *et al.*, 1999; Robertson *et al.*, 1993). MUPs are a multigene family of lipocalins, often excreted in high quantities in urine or other secretions such as saliva (Beynon & Hurst, 2004), prolonging the release of volatile chemosignals from scent marks to attract females to investigate scents over a prolonged period (Humphries *et al.*, 1999). House mice have been extensively used in MUP research due to the amount of

polymorphism shown (Humphries *et al.*, 1999; Logan *et al.*, 2008), even in highly geographically constrained populations where background genetic variation is low (Beynon *et al.*, 2002). MUPs are stable and individual patterns are thought to be consistent throughout a lifetime (Beynon & Hurst, 2004). They are thought to be important in female individual recognition of males (Cheetham *et al.*, 2007) and a reliable source of identification (Beynon & Hurst, 2003; Robertson *et al.*, 1996). Wild mice are likely to excrete a combination of 10 to 15 MUPs in their scent marks and females use this profile to associate with males that are genetically heterozygous at MUP loci (Thom *et al.*, 2008b). MUPs have been suggested as an important mechanism for kin recognition in house mice as relatives are thought to share more MUPs than unrelated females (Beynon & Hurst, 2004; Holmes, 2012; Sherborne *et al.*, 2007). In an experimental setting, male mice were found to react less aggressively towards the odour of a MUP-similar brother than a MUP-dissimilar brother (Hurst *et al.*, 2001b).

MUPs expressed at particular masses have been shown to play specific roles; for example darcin is expressed at mass 18,893 Da by all adult wild-derived males (Roberts *et al.*, 2010), binding and releasing the majority of thiazole (Armstrong *et al.*, 2005), to elicit female attraction and memory to male scent (Roberts et al, 2010; Roberts et al, 2012). It is not yet known however if females express sex-specific MUPs which could be used in competitive signalling (J. L. Hurst, personal communication). Under experimental conditions, mice have been shown to excrete urinary MUPs at concentrations ranging from 10 to 20 mg/ml in males, and 2 to 5 mg/ml in females (Beynon & Hurst, 2004). This can increase three-fold when males and females are engaged in territorial defence (Garratt *et al.*, 2012; Garratt *et al.*, 2011b), resulting in a substantial loss of urinary protein (Gosling *et al.*, 2000; Zala *et al.*, 2008). If MUPs are energetically costly to produce then lower quality individuals may be unable to invest as heavily as high quality individuals, suggesting a reduction in protein output for more subordinate individuals. However, Malone (2002) found that there was no trade off in growth as a response of increasing MUP output in males, suggesting that when food is abundant, male house mice are able to sustain increased protein production.

1.8.5 Scent marking in female house mice

Female scent marking has received relatively little attention in species other than house mice. There are reports of scent marking in golden hamsters (*Mesocricetus auratus*)

(Johnston, 1977), prairie voles (*Microtus ochrogaster*) (Wolff *et al.*, 2002) and wild banded mongooses (*Mungos mungo*) (Jordan *et al.*, 2011). In the latter study the authors found that individuals had distinct scent marks, however there was little evidence that female scent over-marking was related to competition for food or to mediate reproductive suppression (although females that over-marked more frequently were mate guarded by high-quality males and therefore may be more attractive) (Jordan *et al.*, 2011). In house mice there is convincing evidence that females scent mark to advertise breeding status (Hurst, 1990c; Hurst, 1990d), although Hurst (1990c) found that scent marking responses were not related to observed aggression in wild females.

The role of MUPs in female communication is less well known, but the MUP profiles of individual females are still thought to be as robust as that of males (Thom & Hurst, 2004). MUP concentration increases at oestrus in female mice (Stopka *et al.*, 2007) and therefore there is likely to be some natural fluctuation in female urinary protein output throughout the oestrus cycle. As reproductive success may be increased when related females communally rear offspring (Konig, 1994b), females may prefer to nest with females more genetically similar to themselves (Holmes, 2012), resulting in reduced levels of competition. If there is minor variation in MUP expression as a result of a change in social status (and therefore fluctuation in androgen production), then MUPs may play a role in female competition. Additionally, females may also prefer to spend time in proximity to males with a dissimilar MUP profile to themselves (Holmes, 2012; Ramm *et al.*, 2008; Thom *et al.*, 2008a; Thom *et al.*, 2008b), and therefore MUP sharing between males and females is also important when studying mate preference in a species such as house mice.

1.8.6 The role of the preputial/clitoral glands in competitive signalling in wild house mice

The male preputial gland has been studied in a variety of species including primates, carnivores, proboscids, ungulates and rodents (see Bronson & Marsden, 1973; Novotny *et al.*, 1990; Novotny *et al.*, 1999; Novotny & Wiesler, 1999; Zhang *et al.*, 2008a). Preputial glands are specialised sebaceous glands (Noble & Collip, 1941; Orsulak & Gawienowski, 1972), formed of modified sebaceous acini secreting either through the skin or into voided urine (Achiraman *et al.*, 2011a). Scent gland secretions such as farnesenes and squalene are important in communicating species, gender and social status (Kannan & Archunan, 2001). Squalene has been found in saddleback tamarins (*Saguinus fusciollis*) and in male giant

pandas (*Aliuropoda melanoleuca*) and is suggested to be used as a sex pheromone to attract mates (Epple *et al.*, 1979; Zhang *et al.*, 2008a).

Female mice have a relatively smaller gland, called the female preputial or clitoral gland, which is located in a similar position to the male preputial gland, although the function is not well known (Donohoe *et al.*, 1981; Gawienowski *et al.*, 1976; Hayashi, 1979; Thody & Dijkstra, 1978). Clitoral gland secretions are likely to be rich in lipids, which are known to vary throughout the reproductive cycle (Achiraman *et al.*, 2011b). A recent experimental study by Achiraman *et al* (2011a), revealed that the clitoral gland of Wistar rats (a laboratory strain of the species *Rattus norvegicus*) contained up to 23 volatile compounds, similar to those reported in California mice (*Peromyscus californicus*) (Jemiolo *et al.*, 1994) and in Swiss house mice (*Mus musculus*) (Achiraman & Archunan, 2006), and higher than the number of volatiles reported in the house rat (*Rattus rattus*) (Kannan *et al.*, 1998). Farnesol was not detected in Achiraman *et al*'s study (2011a), but Zhang *et al* (2008b) found both farnesene and squalene in female rats. Squalene is also known to increase around the time of oestrus as intact male Wistar rats were found to spend more time self-grooming in close proximity to clitoral gland extracts in an experimental study (Achiraman *et al.*, 2011a). Removal of the clitoral gland is also thought to reduce olfactory attractiveness during ovulation in Wistar rats (Lucas *et al.*, 1982). Oestrogen has been suggested to be the stimulant of sex pheromones from the clitoral gland of the female rat (Donohoe *et al.*, 1981; Gawienowski *et al.*, 1976; Thody & Dijkstra, 1978), while progesterone suppresses release of sex pheromones (Lucas *et al.*, 1982). Together this evidence suggests that the secretions from female clitoral glands may play a role in fertility signalling and that they may be under hormonal control (Achiraman *et al.*, 2011a).

1.9 Thesis overview

Throughout this introduction I have highlighted some of the recent evidence for female competition in a range of species and discussed the important fitness consequences in terms of reproductive success, health and survival. Previous studies have highlighted the costs and benefits of attaining breeding positions in singular cooperative breeding systems and there has also been evidence for reproductive suppression and infanticidal behaviour within communal systems, although the focus has been to compare reproductive output of related and unrelated individuals. Consequently there has yet to be a comprehensive study of the dynamics involved when forming a social relationship with a communal breeding

partner, looking at the physiological costs of competition and how this can affect male mate choice and female reproductive success.

In this thesis I examine the strength of competition between unrelated pairs of wild house mice, investigating the effects of individual characteristics and traits such as body mass, anogenital distance and circulating hormone levels on competitive behaviour (Chapter 3). Chapter 4 focuses on the physiological responses of females in competitive environments, investigating the changes in adrenal responses, reproductive cycles and MUP investment. The impact of female social status on male mate choice and mating behaviour is examined in the subsequent chapter, using a series of experiments to test male preference for female odour and preference when given restricted and free access to females. Reproductive success and maternal behaviour of communally nesting female pairs are then examined in Chapter 6, to determine if more competitive females have an advantage when nesting with a lower-ranking social partner. Finally I conduct a comparative analysis to examine if life history traits are influenced by the potential for competition in communal and cooperative breeding systems, with a particular examination of sexual size dimorphism (Chapter 7).

The combination of behavioural, biochemical and comparative methods used in this thesis illustrate the significance of female competition on reproductive success in a communally breeding species and allow an examination of the evolutionary implications of competition in mammals.

Chapter 2 Methods

This chapter describes the general methods used in behavioural experiments throughout this thesis. Specific details of experimental schedule, assays and statistical analysis are described in more detail in the relevant subsequent chapters.

2.1 Animal housing

House mice were captive bred from an outbred colony, established from individuals captured from populations across the North West of England. All animals were maintained under controlled environmental conditions: temperature 20-21^{o}C, relative humidity 45-65% and a reversed 12:12 hour light-dark cycle, with the dark phase commencing at 08:00 hr.

At weaning (post natal day 24), females were housed in single-sex groups of siblings consisting of 2 to 5 individuals in MB1 cages (45 x 28 x 13 cm, North Kent Plastics, UK). Males were singly housed in M3 cages (48 x 15 x 13 cm, North Kent Plastics, UK). Each cage was lined with Corn Cob Absorb 10/14 substrate and contained paper-wool nesting material (Shredded Nesting International Product Supplier Limited, London, UK). Environmental enrichment was also placed inside the MB1 cages in the form of cardboard tubes (11 length x 5 cm diameter), red plastic mouse houses (15 x 11 x 7.7 cm, Techniplast, NJ, USA) and lid-suspended nest boxes (6.4 x 8.3 x 5.7 cm, MPlex, Otto Environmental, WI, USA). M3 cages contained a cardboard tube and a lid-suspended nest box. Water and food pellets were provided *ad libitum* (Lab Diet 5002, International Product Supplies Limited, London, UK). Handling (for either experimental or husbandry purposes), was conducted under dim red light during the dark phase, using a handling tube (19 cm length x 5 cm diameter, one open end and one end closed with aluminium mesh of 0.5 x 0.5 cm) to minimise potential stress and anxiety (Hurst & Beynon, 2010).

2.2 Identification methods

In order to identify females during routine handling, radio frequency identification tags (RFID) were injected beneath the skin at the nape of the neck. This occurred at least 1 week prior to testing to minimise the influence of any potential stress from handing and the injection procedure on experimental behaviour.

During the various experiments in this thesis it was important to quickly and easily identify subject animals visually to ensure accurate recording of behaviour. Where individuals could be recorded at a relatively close distance (approximately 1 m from the test arena) for a relatively short duration (for example 30 minutes), a temporary, water soluble mark was applied to the tail using a odour-free black marker pen either at the base or tip of the tail. This mark could be clearly seen when recording behaviour using a night vision camera. Marks were applied while the subject was held in a handling tube on the test day.

In order to identify mice when behaviour was filmed continuously under red light in enclosures, a more pronounced and longer-lasting mark was necessary. A number of marking methods have previously been tested by researchers at the MBE group and hair dye applied to the fur was deemed to be the most efficient and effective method of marking, while still adhering to good welfare practice. To apply the dye, subjects were captured in a handling tube and gently restrained by the base of the tail at the open edge of the tube (head and body facing inside the tube). Hair dye (Jerome Russell B-blonde, CO, USA) was mixed using the directions on the packaging and a small amount (approximately 1 cm diameter spot) applied using a small plastic spatula to the subject's fur, either 1 to 2 cm from the base of the tail or on the central dorsal area. Females were then released into a clean laminated medium-density fibreboard (MDF) arena (70 x 60 x 55 cm) containing their home cage but with the lid on to allow them to move freely around the arena and smell their familiar odour from the cage. Subjects could also interact with social partners/sisters through the cage lid during this time. After 20 minutes females were recaptured in a handling tube and gently restrained by the tail. Dye was removed using a cotton wool pad soaked in warm water and fur dried by gently holding a clean, dry cotton wool pad over the wet fur. Females were then returned to their home cage and extra paper wool bedding was added to the cage to encourage nesting behaviour and to absorb any further water from the fur. Females were checked every 45 to 60 minutes (over a 4 hour period) after the hair dye application to ensure that there was no evidence of a skin reaction and that no fur loss had occurred. Hair dye was applied 4 days prior to testing to minimise the potential effects of handling on behaviour.

2.3 Urine collection

Urine was collected from individuals using the recovery method during the dark phase. This involves placing the mouse on top of a transparent MB1 cage with a standard metal

grill lid and placing a second empty opaque MB1 cage base upside down on top of the first cage to prevent the mouse from escaping. Urine passes through the lid and can be collected from the lower cage without any contamination from contact with the mouse. As this method does not involve direct handling of mice (unlike the scruffing method where animals are restrained at the nape), this helps to minimise stress. Urine samples were collected once they were visible on the base of the transparent cage using a Gilson P200 pipette and transferred to a clean 1.5 ml Eppendorf tube before storing at -22^{o}C.

2.4 Controlling for reproductive cycle stage

Housing conditions can result in disruption of the oestrus cycle, particularly if female mice are housed in groups of four or more individuals (Van Der Lee & Boot, 1955; Van Der Lee & Boot, 1956). Therefore subject females were housed in MB1 cages (as previously described in Section 2.1) in groups of 2 or 3 familiar sisters at least 1 week prior to testing.

Hormone levels can fluctuate throughout the reproductive cycle, potentially affecting female behaviour (Achiraman *et al.*, 2011a; Huchard & Cowlishaw, 2011). It is also important that females are sexually receptive prior to mating or during mate choice experiments, as males can detect reproductive stage using odour cues (Achiraman *et al.*, 2010). Therefore all females were brought into oestrus by exposing them to soiled bedding from a number of unrelated males, 72 hours prior to testing (Cheetham *et al.*, 2007; Marsden & Bronson, 1964).

2.5 Testing for reproductive cycle stage

In order to test for oestrus stage, subjects were smear tested using a plastic loop (1 µl soft Copan Innovation Brescia, Italy) which was swept just inside the vaginal opening and cells transferred to a 76 x 26 mm slide (Menzel-Glaser Superfrost, Thermo Fisher Scientific Oy, Vantaa, Finland) with a drop of 0.1 % methylene blue stain. A cover slip (26 x 26 mm) was used before examining the cells using a light microscope at x10 objective (M75, Vickers Instruments Ltd., York, UK). The presence of clustered anucleated cornified epithelial cells indicated that the female was in oestrus (Caligioni, 2009) (see Figure 2.1). During this procedure females were handled using a plastic handling tube (as described in Section 2.1) and gently restrained by holding the base of the tail up towards the outer edge of the tube while the body and head remained inside the tube. The entire process took no more than 5 seconds, after which the females were returned to their home cages for at least

3 hours prior to testing. There were no obvious effects of performing smear tests on the mice at the time of testing and they were not averse to entering the handling tube following the procedure.

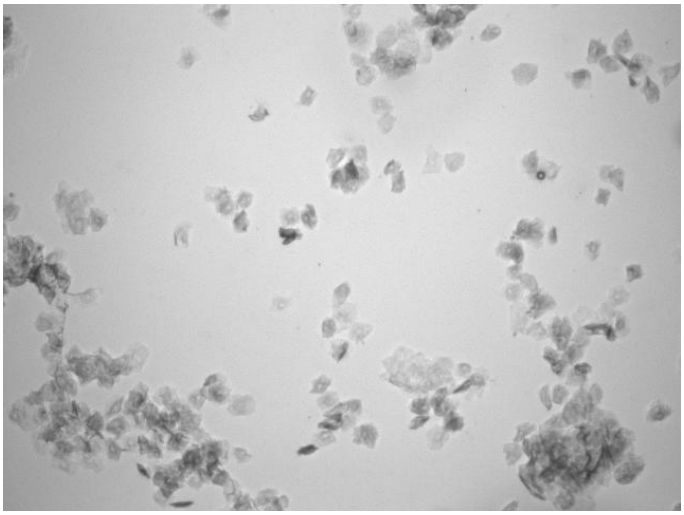


Figure 2.1 – Example of clustered cornified cells viewed at x10 objective on a light microscope. Cornified cells are indicative of the oestrus stage of the oestrus cycle

2.6 Female introduction

In order to record competitive behaviours between female pairs on their first encounter, subjects were introduced in a small arena during the dark phase. Approximately 30 minutes prior to testing, subjects were weighed and habituated to an identical test environment before being transferred to a clean laminated medium-density (MDF) test arenas (70 x 60 x 55 cm) using a separate handling tube for each subject. The test arena contained 1 Perspex sheet (27 x 23 x 0.3 cm) balanced on 4 concrete bricks (20 x 3 x 3 cm) in the centre of the arena and 2 red plastic mouse houses (15 x 11 x 7.7 cm, Techniplast, NJ, USA) in opposite corners of the arena (Figure 2.2). A night-vision camera (Panasonic CCTV camera WV-BP310/B with TV lens WV-LA4R5C3B 1:1,2, 4.5 mm) was attached to a tripod positioned above the test arena to capture behaviours.

Introduction tests lasted for 30 minutes and female behaviour was recorded remotely to DVD (Panasonic video monitor WV-BM1410 and Panasonic DVD/HDD recorder DMR-EX769). The experimenter was present in the room at the time of filming and observed the interactions between females at the opposite corner of the room on a monitor. If competitive behaviour was deemed to be aggressive then the pair was interrupted (see ethical note in Section 2.7). DVDs were watched blind to the identity of the mice at a later date and frequency of competitive behaviours (attack, chase and fight) and submissive behaviours (retreat, submissive posture) were recorded (see Table 2.1 for an ethogram). After each recording session all enrichment equipment and arenas were thoroughly washed in hot, soapy water and repeatedly rinsed to ensure no soap residue remained. Cages were auto-claved and left to air dry.

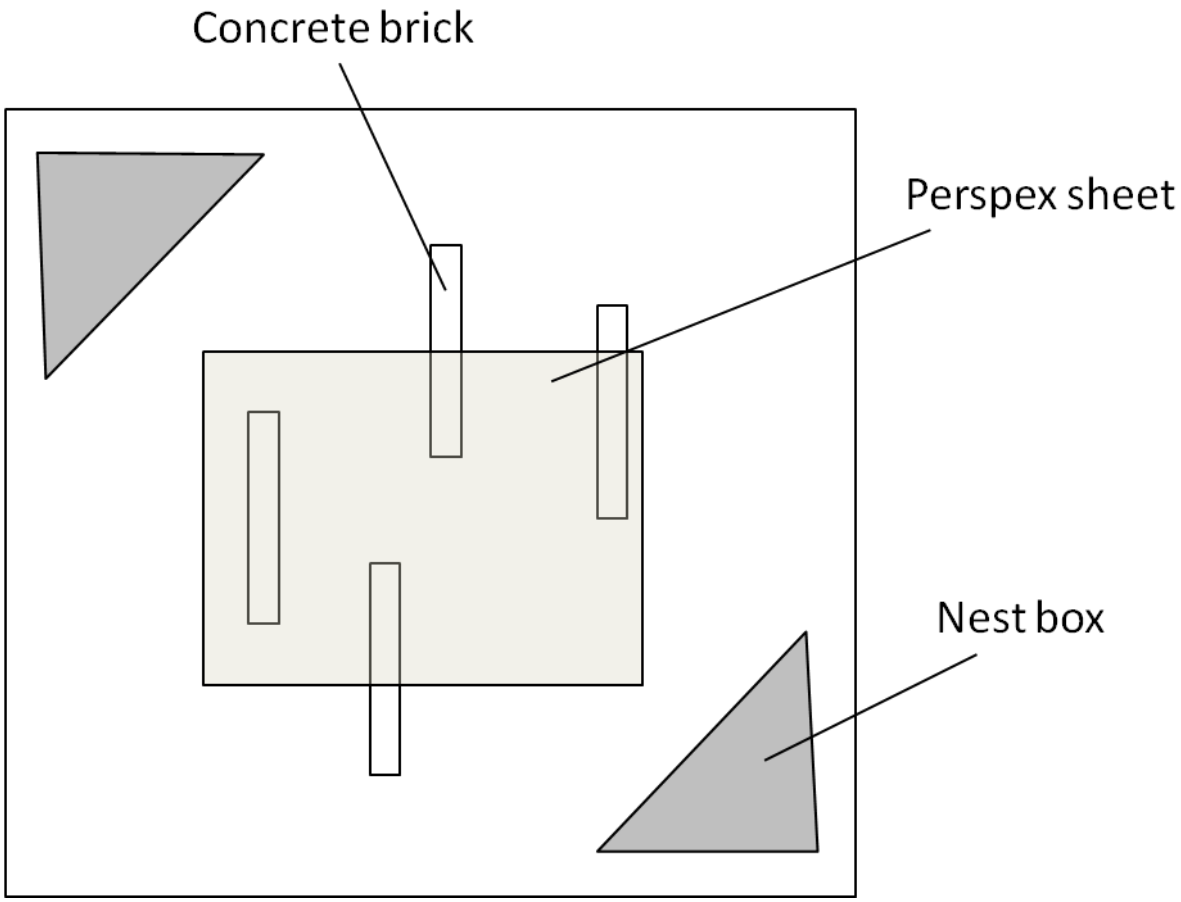


Figure 2.2 – Female competitive introduction arena (not drawn to scale).
Females were released from handling tubes at opposite corners of the arena (top right and bottom left of the diagram) and monitored for excessive aggression. Environmental enrichment was used to provide areas of cover for females during the test. Behaviour was recorded for 30 minutes**.**

Table 2.1 – Descriptions of female behaviour during introduction tests. Behaviour descriptions have been modified from Oortmerssen (1971).

	Behaviour	Description
Investigatory behaviours	**Approach**	Female moves to within half a body length of another individual with nose pointed in their direction.
	Follow	Walking directly behind or slightly beside and parallel to another individual, keeping to the same path as the leading individual.
Aggressive behaviours	**Attack**	Rushing and leaping at an individual with or without kicks/bites.
	Chase	Rapid locomotion to follow the path of a retreating individual, moving at an increased speed from an average walking pace (as in follow).
	Fight	Behaviour performed by 2 individuals when locked together with kicking, biting and wrestling behaviour, usually involves the 2 animals rolling over each another in a rapid movement.
Submissive behaviours	**Retreat**	Following an interaction or approach, rapid locomotion away from another individual, moving at an increased speed from an average walking pace.
	Submissive posture	Sitting upright on 2 legs with front paws close to the body and head upright. Alternatively lying down low with head close to front paws by the floor. Once this position is performed, individuals hold it until the interacting partner moves away or makes contact with them.

2.7 Ethical note

Due to the potential for competitive behaviour in this experiment an ethical rule was applied when females were free to interact with other individuals. Any aggressive behaviour (attack, fight, chase) that exceeded 10 seconds was interrupted by the observer placing a hand over the arena to induce a predatory escape response (where subjects break apart and flee to an area of cover or to the corner of the arena). If the hand placement was not effective then the experimenter clicked their fingers over the arena which usually resulted in the pair retreating to cover. If 3 interruptions occurred over a 30 minute period then the trial was stopped and mice returned to their home cages. During enclosure experiments the same rule was applied every 30 minutes for the first 2 hours of the test.

2.8 Enclosure tests

To explore behavioural interactions between pairs or groups for a longer duration than the introduction trials, subjects were placed into semi-naturalistic arenas during the dark phase (see below for description). Three days prior to the test females were weighed and provided with soiled bedding from unfamiliar and unrelated males, placed inside the home cage of the subject individual. Where male subjects were also used in experiments, they were provided with soiled bedding from the cage of female pairs they were to encounter in the test. This helps to stimulate reproductive behaviour in both sexes and also familiarises the male with female scent to reduce aggression at first meeting (Cheetham *et al.*, 2007).

On the day of testing, subjects were weighed and transferred to melamine enclosures (116 x 58 x 80 cm or 116 x 116 x 80 cm) with their social partner in their MB1 cage with the lid initially closed. Enclosures contained 2 Perspex sheets (27 x 23 x 0.3 cm) and 6 concrete bricks (20 x 3 x 3 cm) and at least 3 cardboard tubes (11 x 5 cm) to provide areas of cover. Females were left to habituate to the test room for 30 minutes before the lid was removed, allowing subjects to freely explore the enclosure arena and interact with one another.

Night vision cameras (as used in Section 2.6) were suspended from brackets above the enclosures and behaviour was recorded continuously to a HDD/DVD recorder in an adjacent room. Observations could be made during the experiment via the monitor to ensure that all individuals were interacting and that no excessive aggression occurred during the trials (see ethical note 2.7). Filming commenced once the lid was removed and continued for the duration of the experiment. Females were checked for signs of injury on

a daily basis over the first 5 days by closing the lid of the cages contained within the enclosures to trap the subjects inside. Cages were then transferred to a handling bin and females were ushered into a handling tube to enable the experimenter to check for fur loss or injury without directly handling the mice.

At the end of the experiment, females were checked for the presence of reproductive plugs (if males had been present) or signs of injury and then urine sampled using the recovery method (Section 2.3). Once urine had been collected, females were transferred to their MB1 home cage with their female social partner. Males were removed from the enclosures at the same time as females and checked for any sign of injury before being transferred to a clean M3 cage and returned to the stock room. All enrichment equipment and arenas were thoroughly washed in hot, soapy water and repeatedly rinsed to ensure no soap residue remained. Cages were auto-claved and left to air dry.

2.9 Post mortem measurements

Following the enclosure arena tests in Chapter 5, subject females were humanely culled to take morphological and physiological measurements post mortem. Animals were initially anaesthetised using a mix of oxygen and halothane on a Compact Anaesthetic Workstation (Model No. AN001, Vet Tech Solutions Ltd, UK) followed by cervical dislocation performed by a member of the technical staff at MBE who held a Home Office licence. Subjects were weighed immediately before dissection commenced and checked for any signs of injury on the fur, face or body. Anogenital distance was measured on 2 separate occasions using callipers to maximise accuracy and check repeatability of measurements. Ovaries and uterine horns were removed and examined for signs of fetal implantation, which appears as a small dark red spot along the uterine horn (Deb *et al.*, 2005). Adrenal glands and clitoral glands were carefully removed and weighed before being photographed against a ruler to provide an additional opportunity for measurement. All tissue was placed in a 1.5 ml Eppendorf tube, labelled and frozen at -22^{o}C.

2.10 Major urinary profile (MUP) peak sharing

In order to determine if female pairs shared MUP type or if MUP profiles changed following competitive experience, MUP mass spectra were analysed. Electrospray ionization mass spectrometry was used to produce the mass profiles of MUPs from urine samples collected on experimental days. Samples were run by Amanda Davidson at the

Protein Function Group, University of Liverpool, UK using a Nanoacquity ultra high performance liquid chromatography system (Waters, Manchester UK) and were processed and transformed to a true mass scale using MazEnt1 deconvolution software (Waters Micromass, Massachusetts, USA).

SpecAlign software (version 2.4.1 http://physchem.ox.ac.uk/~jwong/specalign/) was used to measure the position and intensity of peaks between the mass range of 18600 and 18900 Da (Mudge *et al.*, 2008). Protein peaks from the spectra were normalised to the intensity of the most abundant protein to compare profiles of female pairs to the breeding male. From the resulting MUP spectra a peak profile was established for each individual used in the study (Figure 2.3). If the relative intensity of each peak was greater than 0.15 then it was considered true, as peaks of lower intensity may not represent true proteins (J. L. Hurst, personal communication). Peaks were said to match if both individuals displayed a peak at the same position and matched if the relative intensity difference was within 0.5. This value has previously been used as a measure of MUP peak sharing in other experiments conducted in the Mammalian Behaviour and Evolution research group at the University of Liverpool (Holmes, 2012 unpublished thesis; J. L. Hurst, personal communication).

2.11 Scent mark analysis

In order to assess scent mark frequency of individuals, Benchkote was cut to size and secured to the base of the test arena using double sided tape or wrapped around Perspex tiles and secured with sellotape on the underside (depending on the experimental protocol). Any marks deposited by the test subject are then absorbed onto the Benchkote surface, which was carefully removed at the end of the experiment (surgical gloves were worn at this time to ensure no additional marks were transferred to the Benchkote). Scent marks deposited during the experiment were then analysed by scanning the Benchkote sheets using a Bio-Rad Fluor-S MultiImager with 10 second exposure time (Bio-Rad Laboratories Limited, Hemel Hempstead, UK). Scent mark count, perimeter and area measurements were conducted using the 'Analyse Particles' tool in Image J version 1.45s (http://rsbweb.nih.gov/ij/). Using this software the colour of the visualised scent marks was inverted and brightness, contrast and threshold adjusted to maximise visibility of the scent marks before calculating dimensions and frequency of deposited marks. This data was then transferred to a central spreadsheet for statistical analysis.

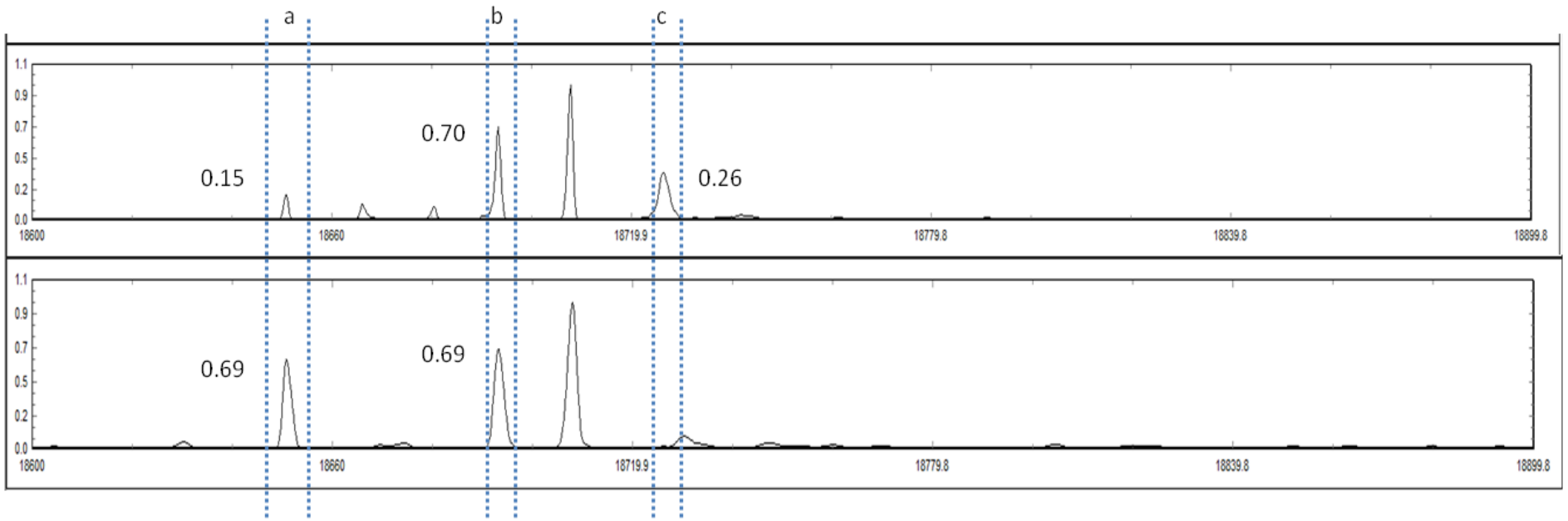


Figure 2.3 – Example MUP mass spectra for female pairs
Relative intensities for the identified peaks are indicated. At mass (a) the females share a peak but do not have matching peaks as the difference in relative intensity is above 0.5. At mass (b) however the females have matching peaks as the difference is less than 0.5. At mass (c) the first female expresses a peak, however the second female does not. Only peaks with a relative intensity above 0.15 were used in the analysis as a true peak.

2.12 Urinary protein analysis

In order to determine protein output of test subjects both before and after competitive interaction with another individual, urine samples were collected using the methods described in Section 2.3 and the concentration of urinary protein measured using the Coomassie plus® protein assay reagent kit from Perbio Science UK Ltd. (Cramlington, Northumberland, UK). Following the protocol of Cheetham *et al*, 2009, each sample was diluted 1:100 with ddH_20, and 100 µl aliquots pipetted in duplicate to a 96 well microtiter plate (Sterilin Microplate F Well 611F96, Thermo Fisher Scientific Oy, Vantaa, Finland). A stock solution of 2 mg/ml BSA was used to generate a standard curve, with concentrations ranging from 0 to 50 µg/ml by diluting the stock solution with ddH_20. Finally 200 µl Coomassie reagent was added to each microtiter plate well. The absorbance of each sample was read at 620 nm in a Thermo Scientific Multiskan FC microplate photometer (Thermo Fisher Scientific Oy, Vantaa, Finland). SkanIt software 3.1 (research edition for Multiskan FC, Thermo Fisher Scientific Oy, Vantaa, Finland) was used to produce a standard curve and the concentration of each sample was calculated by interpolation. All urinary samples collected for each subject were run on the same plate to control for any small differences that may occur between assay runs. As a further measure of standardisation I randomly added two samples that had previously been measured for urinary protein to another plate to ensure that there was no variation between plates.

2.13 Urinary creatinine analysis

Urinary dilution can influence urinary protein concentration and therefore confound values calculated using the above method. Creatinine is produced and excreted in mouse urine at a constant rate according to muscle mass. It is a useful indicator of urinary dilution and is often used to correct for the concentration of protein in mouse urine (Beynon & Hurst, 2004). Therefore an alkaline picrate assay (Sigma Chemicals, UK) was used to measure urinary creatinine values (Cheetham *et al*, 2009). Each sample was diluted 1:50 with ddH_20, and 100 µl aliquots pipetted in duplicate to a 96 well microtiter plate (Sterilin Microplate F Well 611F96, Thermo Fisher Scientific Oy, Vantaa, Finland). A stock solution of 3 mg/dl creatinine was used to generate a standard curve, with concentrations ranging from 0 to 30 µg/ml by diluting the stock solution with ddH_20. Finally 150 µl alkaline picrate reagent (5 ml pictrate colution : 1 ml sodium hydroxide) was added to each microtiter plate well. The absorbance of each sample was read at 492 nm in a Thermo

Scientific Multiskan FC microplate photometer (Thermo Fisher Scientific Oy, Vantaa, Finland). SkanIt software 3.1 (research edition for Multiskan FC, Thermo Fisher Scientific Oy, Vantaa, Finland) was used to produce a standard curve and the concentration of each sample was calculated by interpolation. All samples collected from the same individual were run on the same plate and two random samples were repeated on separate plates to ensure there was no variation between plates.

2.14 Urinary testosterone analysis

Testosterone concentration was measured using enzyme immunoassay methods previously validated for mouse urine (Muir *et al.*, 2001; Munro *et al.*, 1991). Testosterone was obtained from Sigma chemicals, UK, and antibodies to testosterone and corresponding horseradish peroxidise conjugates were obtained from the Department of Population Health and Reproduction at the University of California, USA. NUNC Maxisorb plates were coated in 50 µl of antibody stock diluted 1:10,000 in a coating buffer (50 mmol/l bicarbonate buffer, pH 9.6) then stored at 4°C for a maximum of 7 days. Wash solution (0.15 mol/l NaCl solution containing Tween 20) was then used to rinse away any unbound antibody. Urine samples were diluted 1:10 in phosphate buffer (0.1 mol/l sodium phosphate buffer, pH 7.0 containing 8.7 g NaCl and 1 g BSA). 50 µl of standard or sample then 50 µl of testosterone horseradish peroxidase (diluted 1:25,000) were added to wells. Plates were incubated at room temperature for 2 hours before rewashing with wash solution. 100 µl of substrate solution (Citrate buffer, H_2O_2 and 2,2′-azino-bis) was then added and left to incubate at room temperature until the optimal density of blank wells reached 1.0. Plates were read with a single filter at 405 nm. All samples collected from the same individual were run on the same plate and urinary creatinine was used to correct for dilution of each sample. Two random samples were also added to separate plates to test for variation between plates.

Chapter 3 Characteristics of competitive ability in female house mice

3.1 Chapter overview

Female competition is a relatively overlooked area of evolutionary biology research. There are however examples of female intra-sexual competition to obtain breeding rank, gain access to or control resources or to actively defend young. Differences in age and relatedness between females have previously been suggested to influence reproductive success in wild house mice, however there has been little attempt to measure the influence of physiological characteristics on competitive behaviour. Here I examine a number of characteristics that may influence female competitive ability (age, body mass, reproductive experience, anogenital distance, urinary testosterone and protein levels) and record the amount of competitive behaviour observed when females meet for the first time, in order to calculate a competitive score. Competitive behaviour performed during initial 30 minute trials was consistent in two subsequent trials, reducing in frequency by trial three. Less competitive females spent more time resting under cover, while more competitive females were significantly more active during trials. Reproductively inexperienced female pairs performed a higher frequency of aggressive and submissive behaviours. Female age was only important when predicting submissive behaviour, as younger females were more likely to be submissive. Aggressive behaviour was positively associated with body mass and urinary testosterone concentration; however there were no significant relationships between competitive behaviour frequency and anogenital distance or urinary protein concentration. Finally there was no evidence for a relationship between MUP peak sharing and competitive score asymmetry between female pairs. These findings provide new insights into traits predicting competitive behaviour in female house mice, and may have important implications for group housing female mice in laboratories.

3.2 Female competition for dominance rank

Female-female competition has been relatively overlooked and instead the focus has been to examine male-male competition for mates (Darwin, 1871). However there are now an increasing number of examples of female competition within social mammal species for the purposes of gaining access to resources related to reproduction or for mating

opportunities (see Chapter 1 for examples). Dominance relationships may form between females within social groups if there is a fitness advantage to gaining dominance rank; for example dominant females may benefit from increased reproductive opportunities, or be more likely to gain access to high quality feeding sites compared to subordinate females (Clutton-Brock *et al.*, 1984; Rubenstein & Shen, 2009; van Noordwijk & van Schaik, 1987; van Noordwijk & van Schaik, 1999; Vogel, 2005). In larger groups, high ranking females may also gain more central group positions, minimising the risk of predation such as in long-tailed macaques (*Macaca fascicularis*) (van Noordwijk & van Schaik, 1987).

The benefits of dominance rank may also transfer to offspring. For example in Rhesus macaques (*Macaca mulatta*) offspring of dominant females are more likely to survive longer than offspring born to subordinate females (Meikle & Vessey, 1988), while in chimpanzees (*Pan troglodytes*) the offspring of dominant females have been found to mature faster (Pusey *et al.*, 1997). Maternal rank also determines the dominance position of offspring born into the group in gelada baboons (*Theropithecus gelada*), which results in lifetime fitness benefits for offspring and increased inclusive fitness benefits for the dominant mother (Dunbar, 1980). In groups with limited breeding opportunities females may queue for reproduction or even aggressively compete for rank position (Kokko & Johnstone, 1999). It is therefore often important for females living in competitive social conditions to obtain and maintain dominance rank, in order to increase the chances of successful reproduction (for more examples see Table 1 in Stockley & Bro-Jørgensen, 2011).

3.2.1 Which characteristics can influence the ability to obtain dominance rank in social animals?

Particular traits associated with dominance in males, such as body mass, can also provide females with competitive benefits. Body mass is generally correlated with strength and ability to win contests in a range of species among arachnids (Wells, 1988), fishes (Enquist *et al.*, 1990) and reptiles (Zucker & Murray, 1996). A relationship between body mass and competitive ability has also been demonstrated in mammalian species such as African elephants (*Loxodonta africana*) (Archie *et al.*, 2006), dwarf mongooses (*Helogale parvula*) (Creel & Waser, 1994) and meerkats (*Suricata suricatta*) (Hodge *et al.*, 2008). In some species such as naked mole rats (*Heterocephalus glaber*) and meerkats, females rapidly gain weight following a successful take-over (O'Riain *et al.*, 2000; Russell *et al.*, 2004),

which is likely to be the result of gaining access to high-quality food resources and changes in hormone levels (Clutton-Brock *et al.*, 2006; Russell *et al.*, 2004). Increased body mass and size results in reproductive benefits in terms of the quantity and quality of offspring, with larger females producing larger and heavier litters (Russell *et al.*, 2004); therefore the ability to obtain dominance rank is important for lifetime fitness in some species. Given that body mass appears to be important in obtaining dominance rank and that an increase in body mass can occur as a consequence of maintaining the dominant position, it is important to untangle these effects when measuring important characteristics that may predict the ability to obtain and maintain dominance rank. For example, higher ranking subordinate female meerkats tend to be larger and heavier than other subordinate females and are therefore perhaps better equipped to outcompete rivals when an opportunity for breeding position arises. Once the dominant position is obtained, the female is then likely to further increase in body weight (Hodge *et al.*, 2008; Russell *et al.*, 2004). Therefore female body mass should be measured prior to, during and following competitive take-over situations.

In chimpanzees (*Pan troglodytes*) (Pusey *et al.*, 1997) and mountain goats (*Oreamnos americanus*) (Cote, 2000), dominance rank is thought to be related to age. Female bighorn sheep (*Ovis canadensis*) develop age related social ranks, but this is only evident up to the age of six years, the point in which females reach asymptotic mass (Favre *et al.*, 2008). After this period, ewes are more likely to be dominant if they are larger than their rivals (Favre *et al.*, 2008). As age has a strong positive association with body mass (Creel, 2001), it may therefore be difficult to extrapolate the most important factor in predicting competitive ability, particularly in relatively short lived species with variable adult body mass.

Female competitive behaviour can be influenced by in-utero exposure to androgens (such as testosterone), the levels of which increase with the number of male siblings in polytocous species such as house mice *(Mus musculus domesticus*) (Palanza *et al.*, 2005). Pre natal exposure to testosterone is thought to result in masculinised genitalia and positively influence adult body weight in spotted hyenas (*Crocuta crocuta*) (Frank, 1986; Neaves *et al.*, 1980; Tilson & Hamilton, 1984), which may provide competitive benefits for offspring (Dloniak *et al.*, 2006). However, high levels of androgens may also negatively affect ovarian activity and subsequent fertility (Glickman *et al.*, 1998; Packer *et al.*, 1995), so a trade off in androgen exposure and production may be necessary. High

levels of testosterone may not initiate competitive behaviour per se, but sustains the behavioural response to competition, and can be released in anticipation of competition, resulting in an increased frequency of aggressive behaviours and alertness (Bergmuller *et al.*, 2010; Gleason *et al.*, 2009; Wingfield *et al.*, 1987). Testosterone levels may also increase following competitive interaction for successful winners, which can positively influence future competitive events (Oyegbile & Marler, 2005). In an experiment with bank voles (*Clethrionomys glareolus*), females were more likely to attack intruders in their home cage if they were injected with testosterone prior to the interaction to stimulate the winner effect (Kapusta, 1998). Circulating androgens may therefore serve to protect vulnerable offspring, particularly in species were intruders can be infanticidal (Hrdy, 1979; Maestripieri & Alleva, 1991; Palanza *et al.*, 1996).

Morphological and physiological traits are not the only potential influence on female competitive behaviour. Personality traits may also correlate with competitive behaviour and survival chances (Bergmüller, 2010; Biro & Stamps, 2008); for example 'boldness' increases with winning experience in rainbow trout (*Onchorhyncus mykiss*) (Frost *et al.*, 2007). In a study using field observations of wild chacma baboons (*Papio hamadryas ursinus*), females were defined as 'nice', 'aloof' or 'loners', based on the performance of seven behaviours, which correlated with stress levels and sociality with other group members (Seyfarth *et al.*, 2012). Personality may therefore affect the amount of aggression an individual receives, as 'nice' females in Seyfarth *et al'*s (2012) study, were more likely to signal benign intent by grunting to lower-ranking females, whereas 'loner' females were more likely to avoid other group members, grunting primarily to higher-ranking females. Grooming behaviour can be an signal of submission in some species and therefore negatively correlate with competitive behaviour; for example to gain access to dominant female's offspring in meerkats (Kutsukake & Clutton-Brock, 2006), to reduce aggression in bonnet macaques (*Macaca radiata*) (Silk, 1982), or as a signal of subordinate status in laboratory strains of mice (Gioiosa *et al.*, 2009). Non-aggressive behavioural traits such as boldness or appeasement behaviours may therefore be important signals of competitive potential, however it is important to consider if the personality trait has been the cause or effect of female competitiveness. Without measuring personality extensively before and after competitive situations it is difficult to untangle the importance of traits on obtaining and maintaining dominance rank.

3.2.2 Potential competitive characteristics in female house mice

Only a small number of studies have investigated competitive behaviour between female mice, many of which use laboratory strains (Miczek *et al.*, 2001; Palanza *et al.*, 2005; Rowe & Redfern, 1969; Rusu, 2004; Van Zegeren, 1980; White *et al.*, 1969). Female house mice were previously thought to be non-aggressive except around the time of parturition and/or during the early lactation period (Mackintosh, 1981). However, competitive behaviour has also been observed outside of these periods, which can influence population dynamics of social groups (Hurst, 1987; Palanza *et al.*, 2005; Palanza *et al.*, 1996). As females may become reproductively suppressed through olfactory signals when groups consist of three or more individuals in a nest site (Hurst, 2005), competitive behaviour could increase for reproductive opportunity. Palanza *et al* (2005) suggested that aggression functions to expel same-sex rivals and without it dominance relationships may be unstable between group members. Aggressive behaviour however can also negatively affect reproductive success, leading to reproductive suppression, mating interference or resorption of foetuses in house mice (Lloyd & Christian, 1969; Palanza *et al.*, 2001; Palanza *et al.*, 1996). In-utero exposure to testosterone can result in longer anogenital distance, which has been suggested to positively correlate with aggressive behaviour and attack latency in female house mice (Palanza *et al.*, 2005). If females with more masculinised genitalia and behavioural traits also have higher levels of circulating androgens, then testosterone may be excreted in the urine as a signal of potential competitiveness (as observed in male house mice Malone *et al.*, 2005). Although, high levels of androgens can be costly for females in terms of ovarian activity and fertility (see Chapter 1).

Another important component of scent communication in mice relates to major urinary proteins (MUPs) which are excreted in vast quantities in the urine (Beynon & Hurst, 2004). MUPs have been suggested as an important mechanism for kin recognition in house mice as relatives are thought to share more MUPs than unrelated females (Holmes, 2012; Sherborne *et al.*, 2007). MUP peak profiles may therefore be used as a measure of genetic similarity between individuals (see Chapter 1 for a review on MUPs). As aggression is thought to be reduced between related individuals (Palanza *et al.*, 2005; Rusu, 2004; Rusu & Krackow, 2004), lower levels of competition could be expected between female pairs with relatively similar MUP peak profiles. Familiarity with an individual may also reduce

the amount of aggressive behaviour observed on first meeting in house mice (Latham & Mason, 2004). In the wild, females predominantly interact with familiar sisters and unfamiliar, unrelated individuals (Rusu & Krackow, 2004). In an experimental condition, 80 % of non-sister hierarchies were found to be linear, with one female dominating the other (Rusu & Krackow, 2004). Interestingly, aggression was also higher between females when only one male was present in the nest area compared to three males, suggesting that females were competing for access to the male in this experiment (Rusu & Krackow, 2004). If female house mice are competing for reproductive opportunities, then older females may compete more intensely as there would be limited opportunities to mate in their remaining lifetime compared to younger females. In another experimental study, Rusu *et al* (2004) found that older female house mice dominated their younger siblings in terms of reproductive output, even if the older females were lighter than their sibling. There was however no measure of competitive behaviour performed between females and therefore the difference in reproductive output could not be attributed to infanticide or other competitive behaviours.

Reproductive experience may also be an important factor in deciding how intensely females should compete. Reproductively inexperienced females may compete more intensely for a higher ranking position to enable them to mate when the opportunity arises (e.g. in reproductive queues; Kokko & Johnstone, 1999). However reproductively experienced females may already assume more dominant roles and therefore compete to retain breeding positions (e.g. in meerkats; Hodge *et al.*, 2008).

Of course, success in competition (and consequently reproductive success) could be determined by a combination of the characteristics mentioned above. Weidt *et al* (2008) showed that unrelated females had higher reproductive output if they nested with a 'preferred' partner (i.e. a female with which they previously had a high association score), however there was no reported observation of competitive behaviour in this study. There is therefore a need to investigate which characteristics or traits could be important in predicting competitive ability of potential social partners, as this is likely to strongly influence future reproductive success.

3.2.3 Repeatability of competitive behaviour in mice

Due to the energetic costs and risks of injury associated with competition, individuals benefit from making assessments of their own competitive ability compared to their opponent, usually through display behaviour (Arnott & Elwood, 2008; Parker, 1974). Although fights are likely to initially occur between competing individuals, the frequency should quickly decrease to avoid the risk of injury or death (Parker, 1974), resulting in the formation of dominance hierarchies (Rowell, 1974). Subordinate individuals learn not to challenge dominants due to the risk of losing competitive encounters, while dominant individuals do not have to waste energy or risk injury by re-establishing superiority and instead limit aggression to threat behaviours (Cant, 2011; Rowell, 1974; Wong *et al.*, 2007). As little is known about dominance formation in female house mice, there are no indications of how quickly females may establish social rank. Previous experimental studies of aggressive behaviour in house mice use trial lengths ranging from 15 minutes to 24 hours, and some repeat the trials over a number of consecutive days (Benton *et al.*, 1980; Oortmerssen & Bakker, 1981; Palanza *et al.*, 2005; Palanza *et al.*, 1996; Van Zegeren, 1980). Although aggressive behaviour between females may not be as prolonged and intense as that shown by male mice (Van Zegeren, 1980), it is important to investigate consistency in the direction of aggressive behaviour between female pairs to accurately allocate dominance rank.

3.3 Experimental aims

There has been relatively little work examining the occurrence of competitive behaviour by female house mice or to investigate the characteristics that may influence individuals attaining higher competitive rank. The aim of this chapter is therefore to investigate competitive behaviour of female mice at first encounter and investigate a number of characteristics that may be used to predict female competitive ability. Competitive behaviour is analysed in terms of intensity (i.e. the number of competitive acts performed) and competitive asymmetry between female pairs. For a subset of animals, repeated introduction tests were conducted to test for the consistency and direction of competitive behaviour performed between pairs. As relatedness and age have previously been shown to influence competitive dynamics, I study the behaviour of unrelated females paired with an age-matched partner or with a different aged partner to further examine the effect of age on competitive behaviour. I predict that older females will perform more aggressive behaviour

than younger partners and that older age-matched females will be more competitive than younger age-matched female partners. I also investigate the behaviour of reproductively experienced age-matched and age-difference pairs to determine if reproductive experience influences competitive behaviour. For a subset of animals I record the amount of time females spend resting under cover or outside of cover, as well as activity levels and self-grooming behaviour as measures of boldness and subordination. Finally I investigate the relationship between competitive behaviour at introduction and other physiological measurements such as body mass, anogenital distance, urinary testosterone and protein levels, as well as a comparison of MUP peak profile similarity between female pairs.

3.4 Methods

3.4.1 Animals

Behavioural observations were collected using a total of 84 pairs of female wild house mice under experimental conditions. Females were aged between 3 and 16 months at the time of testing and paired with an unrelated and unfamiliar female that was either matched for age or with an age difference of approximately 3 months (Table 3.1). Females aged 3 to 4 months were used to represent the average youngest age that female mice reproduce in the wild. Females aged 12 to 16 months represent older females that are at the end of their reproductive life. As females may be motivated to compete for reproductive opportunity, females were also matched for previous reproductive experience. Reproductively inexperienced females had previously encountered male odour in their home cage (by adding soiled male bedding), and limited interactions with males four days previously (see Chapter 5). Reproductively experienced females had previously been mated to an unrelated and unfamiliar male and successfully weaned a litter 1 week prior to testing. Prior to testing all animals were housed as described in Chapter 2, Section 2.1.

Table 3.1 – Summary of treatment groups and sample sizes

	Age-matched			**Age-difference**
	3 to 4 mths	**6 to 7 mths**	**12 to 16 mths**	**3 and 6 mths**
Reproductively inexperienced	10 pairs	9 pairs	24 pairs	10 pairs
Reproductively experienced	15 pairs	-	-	16 pairs

3.4.2 Experimental procedure

Subject females were introduced to an unfamiliar and unrelated female social partner in a test arena as described in Chapter 2, Section 2.6. Following a 30 minute habituation period females were weighed and then simultaneously released into the test arena using handling tubes. Competitive behaviour was recorded remotely to DVD and data collected and analysed at a later date.

If females were excessively aggressive and had to be interrupted more than 3 times during the 30 minute test period then the test was stopped and subjects returned to their home cages (see ethical note in Chapter 2, Section 2.7).

3.4.3 Repeated interaction test

Many studies of competitiveness and the establishment of dominance relationships in pairs or groups of male mice use repeated encounter trials between individuals to test for consistency in the direction of competitive behaviour observed. Therefore a subset of female pairs were introduced 3 times to the same social partner over 5 days, with a rest day in between each trial. At the end of the first trial female pairs were transferred to a divided cage to enable limited tactile contact between females between trials. Divided cages were specially designed MB1 cages that were laterally bisected with a Perspex barrier (45 x 13 cm) with a section cut out along the middle covered on either side with aluminium wire mesh (mesh spacing 0.5 cm) to enable limited tactile and visual contact while maintaining full auditory and scent communication. Cages had a specially adapted wire lid to enable water bottles and food pellets to be placed on either side of the mesh barrier. The bottom of each cage was lined with substrate and contained paper wool nesting material as described in Section 2.1. At the end of the third test, female pairs were transferred to a clean MB1 cage (as described in Section 2.1) with a handful of soiled bedding taken from each side of their divided cages to retain some familiar odour and reduce the potential for further aggression. Once again if females were excessively aggressive during the interaction trials and had to be interrupted more than 3 times over the 30 minute test period (see ethical note in Section 2.7), the trial was stopped and the pair transferred to divided cages to enable limited contact.

3.4.4 Urine analysis

Urine samples were collected 4 days prior to testing from each subject female using the recovery method described in Section 2.3. Baseline urinary protein and testosterone levels were then calculated using the assay methods described in Sections 2.12 and 2.14 respectively. Assay results were corrected for urine dilution by dividing the protein or testosterone value by creatinine output analysed using the method in Section 2.13.

Subject urine samples were also analysed to establish if female pairs had similar or dissimilar MUP profiles. MUP mass spectra were analysed using electrospray ionization mass spectrometry and visualised using SpecAlign software as described in Section 2.10.

3.4.5 Data analysis

Competitive behaviours recorded during the experimental trials were watched blind to the identity of subject individuals (see Table 2.1 for an ethogram of behaviours recorded). Each single bout of aggressive behaviour scored +1 point and a single bout of submissive behaviour scored -1 point. Points scored over the 30 minute test period were totalled to produce an overall competitive score. Females with the highest competitive scores were classified as the most competitive female in each pair. Where data did not meet parametric assumptions a log transformation was applied. When data could not be normalised through transformation, non-parametric statistics were used. All analysis was conducted using R Software v2.15.1 (R Development Core Team, 2010) unless otherwise stated.

Consistency of competitive behaviour

A Friedman's test was conducted using SPSS v20 to establish if relative competitive score between female pairs was consistent throughout the trials. Relative competitive scores calculated for the second and third interaction trials were not normally distributed and could not be transformed using conventional methods. A Bonferroni correction (critical value = 0.017) was therefore applied to correct for the number of Wilcoxon tests performed. Aggressive and submissive behaviour frequencies were also compared over the three tests using Wilcoxon tests.

Predictors of competitive behaviour

To test which characteristics may influence the incidence of aggressive and submissive behaviour when encountering an unfamiliar female conspecific, I used generalised linear mixed models with a logarithm link function and Poisson distribution, fitted using the Laplace approximation to restricted maximum likelihood estimation (lmer procedure in the lme4 R package, (Bates *et al.*, 2012). Female pair was included as a random effect to control for non-independence.

Other behaviours

The amount of time that individuals spent in activity (i.e. continuous locomotion), self-grooming, and resting under or outside of cover were recorded for a subset of reproductively inexperienced female pairs. Paired t-tests were used to determine if females differed in the amount of time they spent performing these behaviours, according to age or competitive rank. Finally the relationship between shared MUP peak profiles and competitive score asymmetry was tested using a Spearman's rank correlation test.

3.5 Results

3.5.1 Repeated measures of competitive behaviour

To test for consistency in competitive behaviour in repeated encounter tests, a subset of 24 female pairs were introduced in three tests over a five day period. Median competitive scores for subject females (the female with the highest competitive score within each pair) were not significantly different across the three trials (χ^2 = 4.217, df = 2, p = 0.121). However, competitive score asymmetry within each pair was found to be significantly different across the three interaction tests (χ^2 = 12.851, df = 2, p = 0.002). Post hoc tests indicated that competitive asymmetry was significantly different between the first and third test (Z = -3.089, n = 23, p = 0.002), but not different between tests one and two (Z = -1.903, n = 24, p = 0.057) or between tests two and three (Z = -1.632, n = 23, p = 0.103) (Figure 3.1).

To further explore this finding I investigated whether competitive behaviour was reduced over time for all females. Although the frequency of aggressive behaviour did not significantly change between tests one and two (Z = -1.113, n = 48, p = 0.266), there was a significant reduction in aggressive behaviour frequency between tests two and three (Z = -2.887, n = 46, p = 0.004). However the frequency of submissive behaviour steadily decreased between each test (one to two Z = -3.441, n = 48, p = 0.001; two to three Z = -2.587, n = 46, p = 0.010; one to three Z = -5.047, n = 46, p < 0.001). These results suggest that both aggressive and submissive behaviours were reduced 84 hours after first encountering an unrelated and unfamiliar female. As the frequency of aggressive behaviour was consistent from test 1 to 2 but there was a significant reduction in submissive behaviour, I conducted all further analyses of competitive behaviour using data collected during the first test (i.e. behaviour performed over 30 minutes).

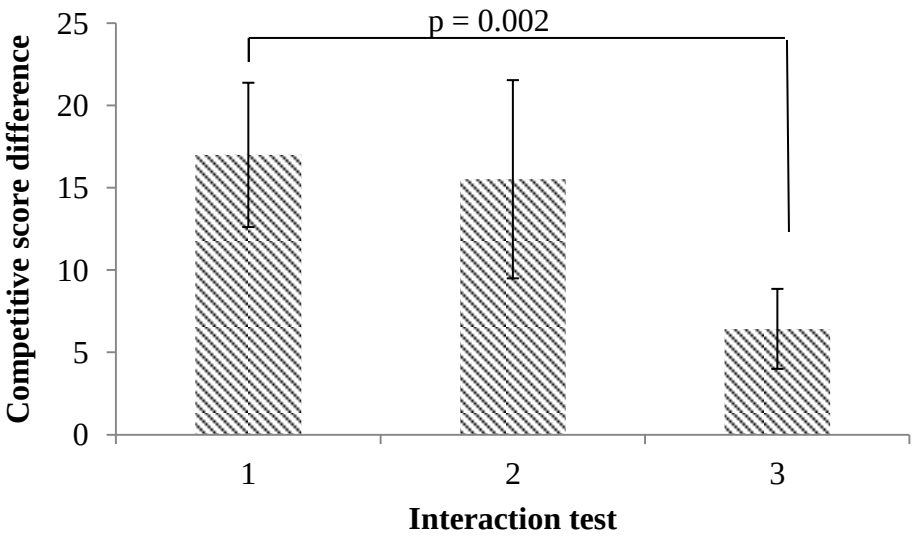


Figure 3.1 – Competitive score asymmetry between older age-matched females across 3 interaction tests (mean ± se). A difference of 0 in competitive score indicates that both females had a similar score during the test.

3.5.2 Influence of individual characteristics on competitive behaviour

Trait effects on aggressive behaviour were tested using a generalised linear mixed model approach. Female mice (n = 168) expressed between 0 and 52 aggressive behaviours (median = 1). Individual body mass and urinary testosterone were both found to have a positive effect on the frequency of aggressive behaviours performed when females were introduced to an unfamiliar and unrelated female conspecific (see Table 3.2b). Reproductively inexperienced females were also more likely to perform a higher frequency of aggressive behaviour (see Table 3.2b). Anogenital distance, urinary protein concentration and age did not have a significant effect on the frequency of aggressive behaviour performed (see Table 3.2b).

Trait effects on submissive behaviour were tested using the same approach as above. Female expressed between 0 and 57 submissive behaviours (median = 5). Individual body mass and age had a negative effect on the frequency of submissive behaviour recorded (see Table 3.3b). Reproductive experience also had a significant effect, with inexperienced females more likely to perform a higher frequency of submissive behaviour (see Table 3.3b). Neither urinary testosterone concentration, anogenital distance nor urinary protein concentration was found to significantly affect submissive behaviour performed (see Table 3.3b).

Only 5 trials were stopped before the end of the 30 minute test period due to excessive aggression (see ethical note in Chapter 2, Section 2.7). Three of these pairs were reproductively inexperienced and in the age difference categories. One pair was reproductively inexperienced and age matched at 6 to 7 months of age. One pair was reproductively experienced and age matched at 3 to 4 months of age.

Table 3.2 – Generalised linear mixed models (GLMMs) to investigate which characteristics predict the frequency of aggressive behaviour recorded during a 30 minute encounter with an unfamiliar and unrelated female conspecific.

	Fixed effects	Coefficient (se)	z-value	p-value	Random effects	Variance (SD)
a) Maximal model	**(intercept)**	-5.151 (1.310)	-3.931	<0.001	Pair	2.935 (1.713)
	Reproductive experience	-1.463 (0.856)	-1.709	0.087		
	Age	0.001 (0.003)	0.303	0.762		
	Body mass	0.377 (0.048)	7.921	<0.001		
	Urinary testosterone	0.007 (0.002)	2.682	0.007		
	Urinary protein	-0.031 (0.023)	-1.316	0.188		
	Anogenital distance	-0.407 (0.436)	-0.933	0.351		
b) Minimal model	**(intercept)**	-5.619 (1.119)	-5.020	<0.001	Pair	2.731 (1.652)
	Reproductive experience	-1.321 (0.528)	-2.500	0.012		
	Body mass	0.385 (0.040)	9.679	<0.001		
	Urinary testosterone	0.006 (0.002)	2.431	0.015		

Table 3.3 - Generalised linear mixed models (GLMMs) to investigate which characteristics predict the frequency of submissive behaviour recorded during a 30 minute encounter with an unfamiliar and unrelated female conspecific.

	Fixed effects	Coefficient (se)	z-value	p-value	Random effects	Variance (SD)
a) Maximal model	**(intercept)**	8.731 (0.749)	11.656	<0.001	Pair	2.935 (1.713)
	Reproductive experience	-0.106 (0.506)	0.209	0.834		
	Age	-0.002 (0.002)	-0.971	0.331		
	Body mass	0.359 (0.029)	-12.347	<0.001		
	Urinary testosterone	0.000 (0.001)	0.036	0.971		
	Urinary protein	-0.021 (0.071)	1.192	0.233		
	Anogenital distance	0.442 (0.318)	1.388	0.165		
b) Minimal model	**(intercept)**	8.526 (0.673)	12.669	<0.001	Pair	1.804 (1.343)
	Reproductive experience	-0.713 (0.340)	-2.095	0.036		
	Body mass	-0.266 (0.022)	-12.274	<0.001		
	Age	-0.003 (0.001)	-3.042	0.002		

3.5.3 Do behavioural traits (such as activity and 'boldness') vary between competitive females during the interaction trials?

Less competitive females spent significantly more time resting under areas of cover during the 30 minute interaction trial compared to more competitive females ($t_{[26]}$ = -3.085, p = 0.005); however there was no significant difference in the amount of time that more or less competitive females spent resting outside of cover ($t_{[26]}$ = 0.529, p = 0.601). More competitive females were active for significantly longer periods of time during the trials compared to less competitive females ($t_{[26]}$ = 2.937, p = 0.007). There was no difference in the time spent grooming between more and less competitive females ($t_{[26]}$ = 0.275, p = 0.786).

When there was an age difference between pairs, younger females spent significantly more time resting under cover than older females ($t_{[9]}$ = -2.580, p = 0.030) and there was a non-significant trend for older females to spend more time resting outside of cover ($t_{[9]}$ = 2.213, p = 0.054). However there was no difference in the time older and younger females spent grooming ($t_{[9]}$ = 1.122, p = 0.291) or differences in periods of activity ($t_{[9]}$ = 0.762, p = 0.466).

3.5.4 Influence of MUP peak sharing on competitive behaviour

A Spearman's rank correlation test revealed no relationship between MUP peak sharing of female pairs and competitive behaviour of more competitive females at first encounter (r^2 = 0.080, n = 54, p = 0.563) or the competitive score asymmetry between female pairs (r^2 = 0.114, n = 54, p = 0.414).

3.6 Discussion

Body mass was highly important in terms of predicting the frequency of aggressive and submissive behaviour when females were introduced to an unfamiliar and unrelated female conspecific. Previous reproductive experience was also highly influential as inexperienced females were significantly more likely to display higher frequencies of aggressive and submissive behaviour. Younger females also performed significantly more submissive behaviour during the introduction trials.

The reduction in competitive behaviours after 84 hours of housing suggests that female house mice establish competitive relationships within this time period. By quickly adopting social rank positions, females avoid the costs associated with prolonged aggression which is beneficial not just to avoid injury, but also to reduce stress from living in an unstable environment (Parker, 1974; Rowell, 1974). This would potentially enable females to successfully reproduce, although there are also likely to be other benefits associated with obtaining dominance rank (see Chapter 1 for examples). Although competitive behaviour was observed between experienced female pairs, reproductively inexperienced pairs were more likely to display relatively higher frequencies of aggressive and submissive behaviours. Inexperienced females had received limited contact with sexually mature males four days prior to the test, which may have enhanced their motivation to compete for mating opportunities.

Behavioural traits such as boldness may also be indicative of competitive rank in female house mice. Less competitive females spent more time resting under cover and there was a trend for older, more competitive females to spend more time resting outside of cover. Although resting in the open may increase the risk of predation in wild conditions (Kurvers *et al.*, 2009), dominant male mice show a preference for higher vantage points to enable them to listen for approaching predators and quickly retreat to cover, or to listen for approaching competitors which they can then attack from above (Gray *et al.*, 2000; Jensen *et al.*, 2003; Mackintosh, 1981). Conversely, subordinate male mice often shelter under cover when living a dominant male territory (Crowcroft & Rowe, 1963). It is therefore reasonable to assume that less competitive females rest under cover to avoid further aggression from the more competitive female. Activity levels can also vary with social rank. Dominant male mice spend relatively large amounts of time patrolling their territories in the wild (Gray & Hurst, 1998; Jensen *et al.*, 2005; Mackintosh, 1981), and in

this experiment more competitive females also spent more time in activity, moving around the edges and central areas of the test arena (personal observation). Even in the absence of highly aggressive behaviour, social rank may therefore be possible to determine through non-competitive behavioural traits.

Although traits such as masculinised genitalia can correlate with competitive behaviour (and therefore ability to obtaining dominant social rank) in spotted hyenas (Glickman *et al.*, 1993), there was no evidence that longer anogenital distance resulted in higher competitive scores for female house mice in this experiment. However urinary testosterone levels were particularly influential in the performance of aggressive behaviours. As testosterone primarily functions to mediate aggression and may fluctuate in response to competitive interaction, it may also be important to investigate changes in testosterone following competition, to determine if particularly aggressive females maintain higher levels of testosterone (Gleason *et al.*, 2009). Behavioural interactions during development in the nest can have long-term impacts on social behaviour during adulthood. For example experience of living in an unstable social environment during rearing can result in behavioural masculinisation in female guinea pigs (*Cavia porcellus*) (Kaiser *et al.*, 2003). Birth spacing in the communal nest can also influence behaviour during adulthood in laboratory mice, with pups born five or seven days apart displaying more affiliative behaviour; conversely pups born three days apart are more likely to attack during social interaction and show anxiety responses in plus maze tests (Branchi *et al.*, 2009). Mice in this experiment were bred in solitary conditions and therefore early social experience could only have been affected by the amount of sibling competition in the nest. Maternal androgens can however influence competitive behaviour of offspring during development; for example, exposure to yolk testosterone can affect the amount of aggressive behaviour expressed by adult house sparrows (*Passer domesticus*) (Partecke & Schwabl, 2008). Therefore measurements of maternal testosterone may be useful when predicting potential competitive behaviour of offspring at sexual maturity.

MUP peak sharing did not appear to significantly influence competition between female pairs, however as females were unrelated to one another the degree of MUP sharing may be low. The major histocompatibility complex is assumed to be the primary determinant of individual recognition in many vertebrate species (Brennan & Zufall, 2006; Brown & Eklund, 1994); however recent studies in house mice have illustrated a more significant effect of MUPs than MHC on individual recognition (Cheetham *et al.*, 2007; Hurst, 2009;

Hurst *et al.*, 2005; Sherborne *et al.*, 2007; Thom *et al.*, 2008a). These experiments were mainly designed to investigate inbreeding avoidance and only more recently has the mechanisms of female social partner choice been investigated. In his unpublished thesis, Holmes (2012) shows that females prefer to nest with other females that have similar MUP and MHC profiles. As MHC was not investigated in this experiment, it may confound any influence that MUP signalling could have on female competition. As MUPs make up the majority of urinary proteins in mice (Humphries *et al.*, 1999) it is possible that MUPs may play a role in competitive signalling through scent marks deposited by females. Trial length and the potential flurry of aggressive behaviour displayed by newly formed pairs may not have given females sufficient time to deposit many scent marks in the arena, or indeed time to make direct contact to investigate the MUP components contained in the scent marks. Therefore scent marking behaviour should be investigated over a relatively longer period in future studies.

Male house mice increase scent marking behaviour in response to winning an encounter with a rival male (Malone *et al.*, 2005). Although females are not thought to scent mark at the same rate as males (Hurst, 1990c), it could be interesting to investigate how scent mark behaviour changes in response to competitive experience. Garratt *et al* (2011b) reported that reproductively experienced females showed a greater investment in MUPs compared to inexperienced females, particularly if they were engaging in territorial defence. Female house mice are thought to use signals in their urine as an advertisement to males (Hurst, 1990d), therefore it is possible that other females also use these signals to determine local competition for mating opportunities. Urinary signals of competition may be relatively costly to produce (Garratt *et al.*, 2012), particularly when combined with the costs associated with gestation and lactation. Urinary protein levels are thought to be increased during the oestrus period (Stopka *et al.*, 2007) and therefore oestrus stage (and female receptivity to mates) could influence protein output. However this also coincides with the time of highest potential conflict with rival females and therefore protein signalling is likely to be particularly important during this time.

Together these findings illustrate the potential for competition between group housed animals, which is likely to impact on the behaviour of test subjects in experimental conditions. It is also important from an animal welfare perspective to ensure that laboratories with stocks of wild house mice take relevant precautions when housing

females in groups, particularly when body mass is variable between unrelated individuals or following reproductive experience.

3.7 Conclusion

Although female competition is being increasingly investigated in a range of species, there is a distinct lack of studies designed to examine competitive behaviour between females and identify the potential characteristics that could be used to predict competitive behaviour performance. There is some suggestion that age and body mass could be important in obtaining dominance rank (and therefore access to breeding opportunities), as well as a few examples of how masculinised behaviour could influence competitive behaviour of female mice. In this study I found that body mass was particularly important in predicting the frequency of competitive behaviours expressed by female house mice when they meet for the first time. Reproductively inexperienced females were also more likely to perform a higher frequency of competitive behaviours during 30 minute trials. Increased urinary testosterone concentration could also be used to predict the potential high level of aggression that female mice may perform when being introduced to an unrelated and unfamiliar female. However there was no evidence that MUP peak sharing between female pairs influenced competitive behaviour observed, nor was there an effect of anogenital distance on competitive behaviour. I suggest that body mass may be a quick and reliable signal of competitive ability that could be used to assess competitive ability of potential female social partners; therefore body mass should be taken into consideration when housing unfamiliar female house mice together in a laboratory setting.

Chapter 4 Physiological responses following competitive female interaction

4.1 Chapter overview

A number of physiological and morphological responses can occur in response to a change in social status following competitive interaction for both males and females. Although communally breeding species are not thought to develop strong hierarchical relationships, there is increasing evidence of competition between females in terms of aggressive behaviour, reproductive suppression and infanticidal behaviour. The results of the previous chapter indicated that female wild house mice develop social ranks when they are housed with a previously unfamiliar and unrelated female social partner. Consequently, in this chapter I investigated the change in a number of physiological responses known to occur on acquisition of dominance rank in species where female competition is evident. Although there was no evidence of immediate body mass changes following competitive interaction, I found that body mass significantly increased for less competitive older age-matched females from the test day to 40 days post interaction, and for both more and less competitive females 7 to 14 days following competitive interaction. Urinary protein output was increased for both more and less competitive females when they were housed with an age-matched partner at 3 months of age, and for more competitive females when housed with a different aged partner, but not when females were matched with a same aged partner at 12 to 16 months of age. A similar pattern was also found for urinary testosterone; although less competitive females did not show a significant increase overall. There was also evidence for enlarged clitoral glands in competitive females compared to stock females, which may have been affected by testosterone production. The average size of scent marks deposited by more competitive females was reduced following competitive interaction, but unexpectedly scent mark frequency was reduced in the presence of a sexually mature male for both more competitive and less competitive females. Oestrus cycles were not found to be significantly affected following competitive interaction, however there was evidence of social stress for females living with a competitive social partner as adrenal glands were significantly larger compared to similarly aged females that had previously been housed with sisters. Together these results illustrate the physiological effects of competition in female house mice, which could have longer term impacts on reproductive success.

4.2 Introduction

Male-male competition is well documented in mammal species due to the prolific number of species that exhibit strong male hierarchical systems. There are a number of morphological and physiological responses that occur in response to changes in social status following competitive male-male interactions. For example, on acquisition of social rank, dominant males may alter their scent marking behaviour to advertise their status and mark their territory (Malone *et al.*, 2005; Rich & Hurst, 1998). However the evidence of physiological change in response to female-female competition is relatively limited due to the lack of studies on female competition. The majority of evidence for female competition can be found in studies of cooperatively breeding species such as meerkats (*Suricata suricatta*) and naked mole rats (*Heterocephalus glaber*), due to the dominance structure shown between female group members (Clutton-Brock *et al.*, 2006; Russell *et al.*, 2004). Investigations into physiological responses to female competition can therefore be compared between dominant breeding females and subordinate non-reproducing females within these species. Communally breeding species such as wild house mice (*Mus musculus domesticus*) are not thought to develop strong social hierarchies between females, however there is increasing evidence for female competition through reproductive suppression by female group members and age related differences in reproductive success (Rusu, 2004). Consequently there is potential for physiological change in response to female competition, particularly when new social groups are formed or social rank is unstable.

4.2.1 Social stress in unstable groups

Social stress can occur when groups are unstable or new individuals are encountered. This results in activation of the sympathetic nervous system, increasing glucocorticoid production in the adrenal system (Sapolsky, 2002). The adrenal cortex is responsible for secretion of mineralcorticoids, sex steroids (androgens, progesterone and oestrogen) and glucocorticoids (cortisol/corticosterone). Glucocorticoids regulate many physiological processes in response to stress by decreasing metabolism of glucose and the immune response, as well as increasing metabolism of proteins and fats. The stress response results in enlargement of the adrenal glands, evidence of which has been found in sexually mature female voles (*Microtus pennsylvanicus*) in response to group size (Christian & Davis, 1966) and in both male and female Norway rats in response to group instability (*Rattus*

norvegicus) (Haller *et al.*, 1999). This suggests that with increasing competition, the stress response of individuals can result in physiological change (Rubenstein, 2007).

If dominant individuals acquire and maintain their rank through low-level aggression and threats to subordinates, then a rank related pattern of elevated glucocorticoids in subordinate females could be expected. However if hierarchies are non-linear and dominant individuals are often challenged by subordinates then the opposite pattern could occur (Creel, 2001; Goymann & Hofer, 2010). In many non-cooperatively breeding species, subordinate females are thought to have higher glucocorticoid levels than dominant individuals (Creel, 2001). For example, evicted female meerkats show elevated glucocorticoid levels due to aggression directed towards them by the dominant female (Young *et al.*, 2006). The social environment of reproductively mature females is therefore crucial for successful breeding and for offspring development (Kaiser & Sachser, 2009).

In an experiment with guinea pigs (*Cavia aperea porcellus*), Kaiser *et al* (2003) found that females living in unstable social environments showed behavioural masculinisation, increased testosterone output and adapted androgen receptors. Their offspring showed delayed development of the adrenocortical system, with females developing masculinised behaviour and brain development, while males showed a less pronounced expression of male typical behaviour (Kaiser *et al.*, 2003). This response is thought to provide offspring with competitive and reproductive advantages in high-density environments (Kaiser & Sachser, 2009), however for females this reproductive advantage is lost in later life (Kaiser & Sachser, 2005). Male offspring born into high-density environments only receive aggression from dominant males if they developed male typical aggressive behaviour early, so by delaying maturation and avoiding injury, these males have the best chance of gaining alpha status in the future (Kaiser & Sachser, 2009).

4.2.2 The influence of androgens in competitive conditions

The study of social status and androgens (testosterone in particular) has predominantly been examined in relation to male competition, however as testosterone is produced in the adrenal glands, ovaries and placentae of adult female rodents, androgen levels could be useful indicators of female competition (Zielinski & Vandenbergh, 1991). Indeed, female mice are thought to have increased body mass and reproductive advantages in high density environments if they were exposed to higher levels of pre natal testosterone (Vom Saal,

1978). Therefore androgen exposure could provide a distinct advantage in competitive environments.

Testosterone is not thought to initiate aggressive behaviour, but instead sustains the behavioural response to competition over a period of time (Wingfield *et al.*, 1987). Testosterone may also increase in anticipation of competition, preparing the individual to engage in aggressive interaction when it is necessary (Gleason *et al.*, 2009). Following successful male-male competitive interaction testosterone levels often increase in California mice (*Peromyscus californicus*) (Oyegbile & Marler, 2005). This is referred to as the 'winner effect', which has been shown to increase the chances of success in future competitive situations (Oyegbile & Marler, 2005). An influx of testosterone can also be rewarding for individuals, resulting in conditioned place preference for locations that have been previously successful for female Syrian hamsters (*Mesocricetus auratus*) (Meisel & Joppa, 1994) and a strain of laboratory mice (Martínez *et al.*, 1995).

Androgens have also been linked to changes in the preputial gland of males. Subordinate males in high-density populations reduce the production of farnesenes from their preputial glands, resulting in the gland regressing, becoming smaller than that of dominant males (Bronson & Marsden, 1973; Hayashi, 1986). Under experimental conditions, the preputial glands of wild house mice were found to be significantly heavier when males were involved in territorial defence compared to isolated males (Garratt *et al.*, 2012). There is also evidence of similar effects of competition on the size of the female clitoral gland. In an experimental setting Zielinski and Vandenbergh (1991) found that female wild house mice with artificially increased testosterone had significantly larger clitoral glands and reduced uterine mass seven weeks after treatment. If female clitoral glands function in a similar way to male preputial glands, then social status may influence their function, resulting in increases in size and/or mass.

4.2.3 The role of body mass and age in female competition

Dominant females tend to be larger and/or heavier than subordinate females in species such as dwarf mongooses (*Helogale parvula*) and meerkats (*Suricata suricatta*) (Clutton-Brock *et al.*, 2001a; Creel, 2001). In these species, females live in highly organised social groups, with dominant females monopolising reproduction. As a consequence, the potential for female competition is high and physiological changes occur as a result of

changes in social status (Clutton-Brock *et al.*, 2006). Although female mammals can show increased body mass and size with increasing age, Russell *et al*, (2004) found that dominant female meerkats gained weight rapidly on acquisition of alpha status and had wider skulls than subordinate females, independent of age, suggesting that social rank was responsible for this physiological change. In the naked mole rat (*Heterocephalus glaber*), breeding females show an increase in body size on acquisition of alpha rank, which is achieved by elongation of the lumbar vertebrae (O'Riain *et al.*, 2000). Increases in body size could be under hormonal control, as oestrogen and progesterone have the greatest impact on bone growth (O'Riain *et al.*, 2000). However this is an extreme physiological response and there is little evidence of bone growth occurring in response to female competition in other mammalian species.

Increased female body mass and body length can provide reproductive advantages in terms of litter size at birth and weaning weight (Russell *et al.*, 2004). Increases in size are also an advantage when defending dominance status, as body mass strongly predicts competitive ability and performance of competitive behaviours in meerkats (*Suricata suricatta*) (Hodge *et al.*, 2008) and in house mice (Chapter 3). Subordination however usually results in weight loss or slowed weight gain (for example in rats, Barnett, 2009). Increased body mass of subordinate individuals can influence the amount of aggression they receive from dominant females as they are more likely to compete for dominance position, and be successful (Clutton-Brock *et al.*, 2006).

In high density populations where females queue for reproductive opportunities, maturing females would need to compete with relatives from previous generations (Gerlach, 1990). In a communally breeding species, Rusu *et al* (2004) found that older sisters achieved dominance and spatially excluded younger sisters from nest sites, even when older females were lighter. As age of social partners has previously been shown to be an important predictor of submissive behaviour performed between unrelated and previously unfamiliar female pairs of wild house mice (Chapter 3), it is possible that physiological change could be more pronounced between social partners that vary in age and/or body mass.

4.2.4 Scent marking to advertise competitive ability in rodents

In rodent species, scent marking behaviour is an important part of competitive signalling (Johnson, 1973; Malone *et al.*, 2005; Rich & Hurst, 1998). The number of scent marks

deposited and the total area covered has also been shown to correlate with male competitiveness (Drickamer, 2001; Malone *et al.*, 2005) and therefore scent mark frequency is likely to increase following acquisition of dominance rank. Shape of scent marks is also an important predictor of competitiveness with dominant males producing more 'streaky' marks (Malone *et al.*, 2005), while subordinate males 'pool' their urine in a confined area (Desjardins *et al.*, 1973). Defeated males also reduce their rate of scent marking in the presence of a dominant male's marks (Desjardins *et al.*, 1973), which is thought to increase the chance of being tolerated in a dominant male's territory (Hurst *et al.*, 2001b). Dominant individuals repeatedly deposit scent marks in their territory area over several hours (Desjardins *et al.*, 1973; Hurst & Beynon, 2004). Replenishing scent marks maximises freshness, which has previously been shown to influence female mate choice (Hurst *et al.*, 2001a). Females use male scent marks to assess relative competitive ability and consequently their potential quality as a mate (Rich & Hurst, 1998; Rich & Hurst, 1999). The function of female scent marking behaviour is thought to be an advertisement for breeding status (Hurst, 1990c; Hurst, 1990d), although very little work has focused on this behaviour in females.

Male mouse urine contains numerous components that mediate aggression between males, namely thiazole, brevicomin, α-farnesene and β-farnesene (Hurst & Beynon, 2004; Novotny *et al.*, 1990). Farnesenes are also produced in the preputial gland, which is situated close to the urethra, enabling gland secretions to be distributed simultaneously with urine marks (Novotny et al. 1990). The preputial gland of dominant males can be twice as large as those of subordinate males (Novotny *et al.*, 1990), resulting in high physiological demands (Malone *et al.*, 2005). Farnesenes provide additional information on social status and are thought to be used in conjunction with volatile and involatile signals in the urine (Hurst & Beynon, 2004; Novotny *et al.*, 1990). As a result of competition between males, dominant individuals have heavier preputial glands compared to subordinate males, as a result of increased production of farnesenes (Novotny et al. 1990). Female mice have a smaller equivalent, the clitoral gland, although the function of this gland is not yet clear (Achiraman *et al.*, 2011a; Hayashi, 1979; Lucas *et al.*, 1982). As female house mice compete on first meeting (Chapter 3), it is possible that scent marking behaviour will change as a consequence of social status. Scent marking is also likely to be more pronounced in the presence of a male if the function is to advertise dominance status (Hurst, 1990c).

4.2.5 The role of major urinary proteins in competitive signalling

Urine contains high concentrations of protein, 99% of which are Major Urinary Proteins (MUPs), which have a high affinity for thiazole and brevicomin (Humphries *et al.*, 1999; Robertson *et al.*, 1993). If MUPs are energetically costly to produce then lower quality individuals may be unable to invest as heavily as high quality individuals. For example, in an experiment with house mice Malone (2002) found that there was no trade off in growth as a response of increasing MUP output in males when food was abundant, suggesting that dominant males were able to sustain protein production. Males produce MUPs at approximately three times the rate of females (Hurst & Beynon, 2008), with specific MUPs such as darcin (18893Da) involved in female attraction (Roberts *et al.*, 2010). Male age is also thought to influence MUP concentration in house mouse urine, with older males producing a lower concentration of MUPs (Garratt *et al.*, 2011a). Testosterone output is also thought to positively correlate with MUP excretion (Rusu *et al.*, 2008), and therefore if an increase in testosterone occurs in dominant individuals following competitive interaction, an increase in MUP concentration may also occur. The role of MUPs in female communication is less well known. The overall MUP profile of an individual is stable throughout life (Hurst *et al.*, 2001b), although urinary MUP concentration in females has been shown to fluctuate throughout the oestrus cycle, which may help to signal breeding status (Stopka *et al.*, 2007). It would therefore be interesting to examine how the relative intensity of MUP peaks expressed by individuals changes following competitive female interaction, in conjunction with the change in overall urinary protein concentration.

4.2.6 Experimental aims

There is potential for a number of physiological responses to occur following female competition, however to date there have been no studies examining this. In this chapter I therefore aim to identify which (if any) occur in wild house mice, and measure the strength of the change(s). Following competitive female interaction trials (described in Chapter 2), I measure changes in body mass, urinary testosterone and urinary protein. For older age-matched females I investigate the effects of competition on reproductive cycle length. I also look at the differences in female scent marking behaviour in response to male presence, and changes in relative intensity of MUP peaks. Finally I compare the size and weight of adrenal glands and clitoral glands of females post mortem, to determine if

competitively housed females have significantly larger and/or heavier glands compared to similarly aged females previously housed with sisters.

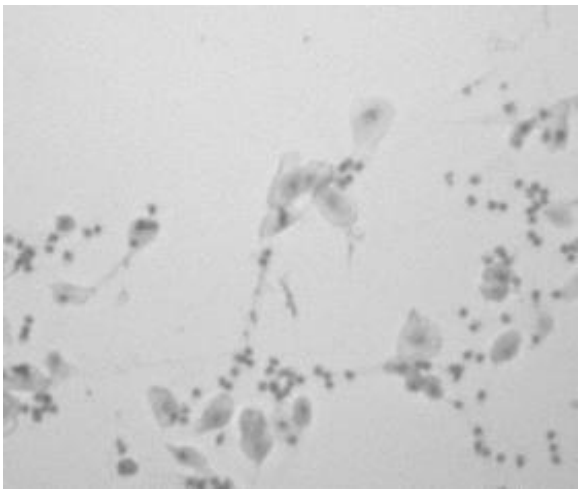
4.3 Methods

Female body mass was recorded on the day of competitive female interaction and 4 days following from 54 pairs of wild house mice during 2 separate experiments conducted in Chapters 5 and 6 of this thesis (see Table 4.1). Further weight measurements were taken for 24 pairs of reproductively inexperienced females 4 days prior to competitive female interaction, and 14 days and 40 days following interaction (see Table 4.1). Urinary protein and testosterone was measured by collecting urine using the recovery method (described in Chapter 2, Section 2.3). Testosterone output tends to be relatively stable across the oestrus cycle (deCatanzaro *et al.*, 2004; Nubbemeyer, 1999), and therefore any changes detected in urinary levels could be reasonably attributed to competitive female interaction. The method for competitive female interaction is described in Chapter 2, Section 2.6 and protocols for analysing urinary testosterone and protein are described in Chapter 2, Sections 2.14 and 2.12.

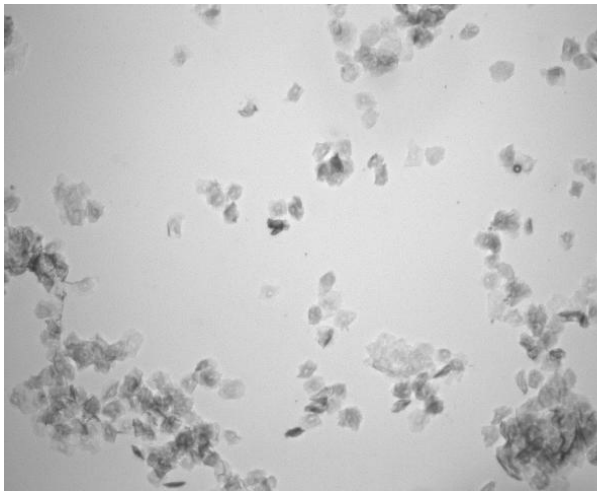
Oestrus cycle patterns were also analysed for 12 female pairs on 3 occasions prior to competitive female interaction and for 23 female pairs on 5 occasions post competitive female interaction with a 2 to 3 day interval (see Table 4.1). Using the methods described in Chapter 2, Section 2.5, cells were taken from females using a plastic loop and transferred to glass slide to examine under a light microscope. Oestrus stage was recorded based on the types of cells observed (Caligioni, 2009) (Figure 4.1). The presence of clustered anucleated cornified cells indicated that the female was in oestrus (Figure 4.1b).

Female scent marking behaviour was measured 4 days prior to and 2 weeks after competitive female interaction as part of the experiment designed to test male preference for age-matched competitive female pairs aged between 12 and 16 months (see Table 4.1). The frequency and average size of scent marks deposited by females during the 30 minute habituation period and 60 minute test was recorded by lining each female's cage with Benchkote to enable any scent marks to be absorbed. The Benchkote was removed from the cage at the end of the experiment and scanned to visualise each mark and analysed using Image J software (as described in Chapter 2, Section 2.11).

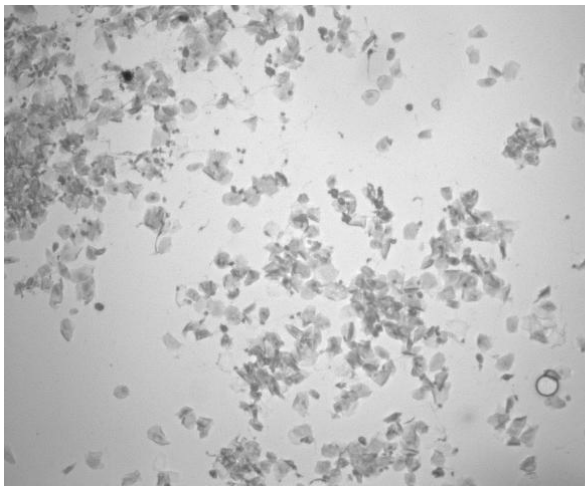
a) pro-oestrus



b) oestrus



c) metoestrus



d) dioestrus

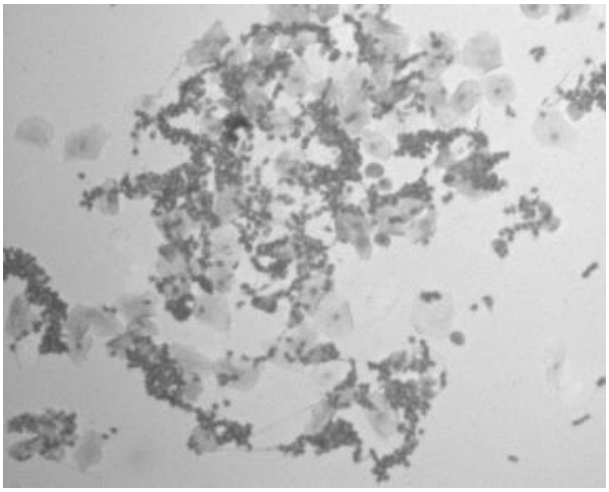


Figure 4.1 - Photomicrographs of stained vaginal cells to identify oestrus stage.
If cells predominantly consisted of nucleated epithelial cells then females were in pro-oestrus (a), whereas clustered anucleated cornified cells indicated females were in oestrus (b). Metoestrus can be identified by the presence of leukocytes, cornified and nucleated epithelial cells (c), whereas leucocytes predominate in dioestrus (d).

Table 4.1 – Summary of treatment groups and sample sizes.
Group A – Reproductively inexperienced, aged matched 12 to 16 months (n = 24 pairs)
Group B – Reproductively experienced, age matched 3 to 4 months (n = 15 pairs)
Group C – Reproductively experienced, age difference 3 and 6 months (n = 16 pairs)
(all sample sizes as above unless otherwise stated)

Weight				**Oestrus cycle length**		**Scent marking**		**Urinary protein/testosterone**		**Post mortem measurements**
4 days pre intro	**Day of intro**	**4 days post intro**	**>4 days post intro**	**Pre intro**	**Post intro**	**4 days pre intro**	**14 days post intro**	**Pre intro**	**4 days post intro**	**Approx 9 to 12 weeks post intro**
Group A	Groups A, B, C	Groups A, B, C	Group A (n = 23)	Group A (n = 12)	Group A (n = 23)	Group A (n = 24)	Group A (n = 23)	Groups A, B, C	Groups A, B, C	Group A (plus additional females from stock population n = 12)

After the experimental period older aged-matched female pairs (see Table 4.1) were examined post mortem to measure the length and weight of the adrenal and clitoral glands, and to examine reproductive organs for abnormalities such as ovarian cysts (as described in Chapter 2, Section 2.9). If a female died before the end of the experiment and their body was stored in the freezer by technician staff prior to dissection, then dissected glands were not included in this analysis due to the potential affect of storage on gland weight and size. A further 12 females aged between 12 and 17 months were also examined post mortem to remove and measure adrenal and clitoral glands for comparison with competitive females. These additional females had previously been housed with their sisters from the age of weaning and were taken from the general stock population at the Mammalian Behaviour and Evolution Group, University of Liverpool.

4.3.1 Data analysis

All data was analysed using SPSS v20 and graphs were produced in Microsoft Excel 2010. Where data could not be logarithmically transformed to meet parametric assumptions, non-parametric tests were used. One female from the age-matched (12 to 16 month) treatment group died before the post competitive samples could be taken and therefore sample sizes were reduced to 23 for the less competitive female analysis. If a value for urinary protein or testosterone could not be obtained from urine samples, then the relevant female was also eliminated from the analysis.

4.3.1.1 Correcting for urinary dilution

Creatinine is a by-product of muscle metabolism and is excreted in the urine at a constant rate (Beynon & Hurst, 2003). Animals with high muscle mass excrete more creatinine in their urine and therefore urinary creatinine output is used to correct for urine dilution when analysing other components such as protein or testosterone (Beynon & Hurst, 2004; Cheetham *et al.*, 2007). However if muscle mass changed in between sampling periods then creatinine cannot be used to correct for urine dilution. I therefore tested if urinary creatinine changed from pre to post competitive interaction for more and less competitive females (urinary creatinine measured using methods described in Chapter 2, Section 2.13). Overall there was an increase in urinary creatinine for more competitive females ($Z = -2.589$, $n = 50$, $p = 0.010$) and a trend for increased creatinine for less competitive females ($Z = -1.899$, $n = 54$, $p = 0.058$) suggesting that muscle mass increased following

competitive interaction. Therefore all protein and testosterone results reported in this chapter use unadjusted values.

4.3.1.2 Statistical tests

i) Body mass

Body mass changes were investigated for all subject females from the day of competitive female interaction to 4 days afterwards (this was classified as a short term response). As older age-matched females (12 to 16 months) were used in a relatively long term experiment, I also measured body mass on a further 3 occasions when females were known to be in oestrus (4 days prior to competitive female interaction and 14 and 40 days following interaction), which was classified as a long term response. Changes in body mass for more and less competitive females were tested using repeated measures general linear models (GLM) for both long and short term sampling periods, with competitive score as a covariate to examine the relationship between competitive behaviour performed and body mass response.

ii) Urinary protein and testosterone concentration

Wilcoxon tests were used to compare urinary protein and testosterone output before and after competitive female interaction for more and less competitive females. Spearman's rank correlation tests were used to look for correlations between urinary output change and female age and competitive score. Paired t-tests were used to compare changes in body mass and urinary protein for older and younger females within the age-difference treatment group, while Wilcoxon tests were used to compare changes in urinary testosterone.

iii) Scent marks

Scent mark frequency and average size of marks was calculated using the methods described in Chapter 2, Section 2.11. Changes in scent mark frequency and size were analysed using repeated measures GLMs with female age, competitive score, changes in body mass, urinary protein and urinary testosterone added to the models as covariates. If there was no relationship between scent mark frequency/size and any of the covariates, then each covariate was removed until either the minimal model was reached or only significant relationships remained.

iv) Adrenal and clitoral glands

Wilcoxon tests were used to compare the weight and length of adrenal and clitoral glands recorded post mortem for more and less competitive females. Mann-Whitney U tests were used to compare gland measurements between experimental females and similarly aged stock females that had been previously housed with sisters. Gland weight and length was corrected for female weight by dividing gland measurement by body mass at the time of dissection.

v) Oestrus cycles

Oestrus cycles were analysed to determine if cycle length altered for more and less competitive females following competitive interaction. Average cycle lengths were calculated using data collected for 3 cycles for pre-competitive and 3 cycles for post-competitive interaction stages. A Wilcoxon test was then used to compare average cycle length for pre and post competition stages for more and less competitive females. A second analysis measured the degree of oestrus cycle synchronicity between female pairs and competitive behaviour performed during the interaction tests. Oestrus stages were coded as follows: 1 at pre-oestrus, 2 at oestrus, 3 at metoestrus, and 4 at dioestrus (with females moving through stages 1 to 4 sequentially and then returning to stage 1). The difference in oestrus stage was then calculated for each pair on 3 occasions following competitive interaction. For example if the most competitive female was in dioestrus and the less competitive female was at pre-oestrus they were 1 stage away from each other in the cycle and therefore would receive a score of 1. If females were synchronised (i.e. at the same stage in the oestrus cycle) then they received a score of 0. A Spearman's rank correlation test was then conducted using competitive score difference between female pairs and the difference in oestrus cycle stage following competitive interaction.

vi) MUP profiles

Finally MUP profiles were analysed to determine how (and if) the relative intensity of the peaks changed following competitive female interaction. MUP spectra were analysed using methods described in Chapter 2, Section 2.10 and the relative intensity of peak heights were measured at both pre and post competitive interaction stages. The change in intensity for each peak expressed by an individual was then totalled, resulting in a value to represent overall change. If females reduced the total intensity of peaks following competitive

interaction the score was negative, while a positive score indicated that the intensity of peaks had increased overall. This score was then compared between more and less competitive females using a paired t-test. The data were also examined to determine if the mass of the most dominant peak for each female differed following competitive interaction.

4.4 Results

4.4.1 Short term effects of female interaction on body mass

4.4.1.1 More competitive females

Body mass did not significantly change for more competitive females following competitive interaction ($F_{[1,51]} = 0.153$, $p = 0.697$). There was however a positive, but non-significant relationship between competitive score and post introduction body mass ($F_{[1,51]} = 3.342$, $p = 0.073$), suggesting that more competitive females tend to gain more weight following competitive interaction when they had higher competitive scores. The results of the repeated measures test also suggested that there were significant differences between age groups ($F_{[2,51]} = 3.550$, $p = 0.036$) On further investigation I found non-significant trends for weight differences between the different aged group and the same aged groups (young, $p = 0.060$; old, $p = 0.078$; see Figure 4.2). There was also a non-significant interaction between body mass change and age groups ($F_{[2,51]} = 2.418$, $p = 0.099$). By analysing age groups separately, I found that competitive females appeared to gain weight following interaction if they were paired with a different aged partner, although this effect was not significant ($F_{[1,14]} = 4.026$, $p = 0.065$). There was however little change in body mass for females paired with an age-matched partner at 3 months ($F_{[1,13]} = 0.616$, $p = 0.447$).

4.4.1.2 Less competitive females

Following competitive interaction, body mass did not significantly change for less competitive females ($F_{[1,50]} = 0.321$, $p = 0.573$). There was no relationship between body mass and competitive score ($F_{[1,50]} = 0.253$, $p = 0.617$) or age group ($F_{[2,50]} = 0.844$, $p = 0.436$). There was also no interaction between body mass change and age groups ($F_{[1,51]} = 1.902$, $p = 0.160$).

4.4.1.3 Body mass change between more and less competitive females

Body mass change was not significantly different between more and less competitive females in the age-difference group ($t_{[15]} = -0.299$, $p = 0.769$), age-matched group at 3 to 4 months ($t_{[14]} = -1.698$, $p = 0.112$) or age-matched group at 12 to 16 months ($t_{[22]} = -0.765$, $p = 0.452$; Figure 4.2).

4.4.2 Longer term effects of female and male interaction on body mass

Older aged-matched females (12 to 16 months) were weighed at various intervals from 1 week prior to competitive female interaction to 8 weeks following interaction, as part of a long term experiment. Females were in oestrus at weighing points and therefore any fluctuations in weight detected during analysis could not be attributed to the oestrus cycle stage.

Prior to competitive interaction tests, females were given limited contact with a male through a mesh barrier. Body mass was measured immediately before male interaction and 4 days later when competitive female interaction took place. Females that would be defined as more competitive during female interaction trials gained a significant amount of weight in the period between male and female interaction ($F_{[1,20]} = 6.285$, $p = 0.021$), however there was no significant difference in body mass for females that would become less competitive ($F_{[1,20]} = 0.214$, $p = 0.649$) (see Figure 4.3). There was no significant relationship with competitive score and changes in body mass for more competitive ($F_{[1,20]} = 0.948$, $p = 0.342$) or less competitive females ($F_{[1,20]} = 0.001$, $p = 0.980$) during this period.

Body mass was found to significantly change between the weighing points for less competitive females ($F_{[2,39]} = 7.387$, $p = 0.002$) and there were non-significant changes between weighing points for more competitive females ($F_{[2.2,43.8]} = 2.786$, $p = 0.068$). Although body mass did not significantly change between interaction and four days post interaction (Sections 4.4.1.1 and 4.4.1.2), both more and less competitive females gained a significant amount of weight between 4 days post interaction and 14 days post interaction (more competitive $F_{[1,20]} = 8.960$, $p = 0.007$; less competitive $F_{[1,20]} = 0.479$, $p < 0.001$; see Figure 4.3). Body mass did not significantly change from 14 days post interaction to 40 days post interaction for more or less competitive females (more competitive $F_{[1,20]} = 0.757$, $p = 0.394$; less competitive $F_{[1,20]} = 0.479$, $p = 0.497$). Body mass did not significantly change from the day of competitive female interaction to 40 days post for more competitive females ($F_{[1,20]} = 0.448$, $p = 0.511$), however less competitive females showed a significant increase during this time period ($F_{[1,20]} = 7.571$, $p = 0.012$).

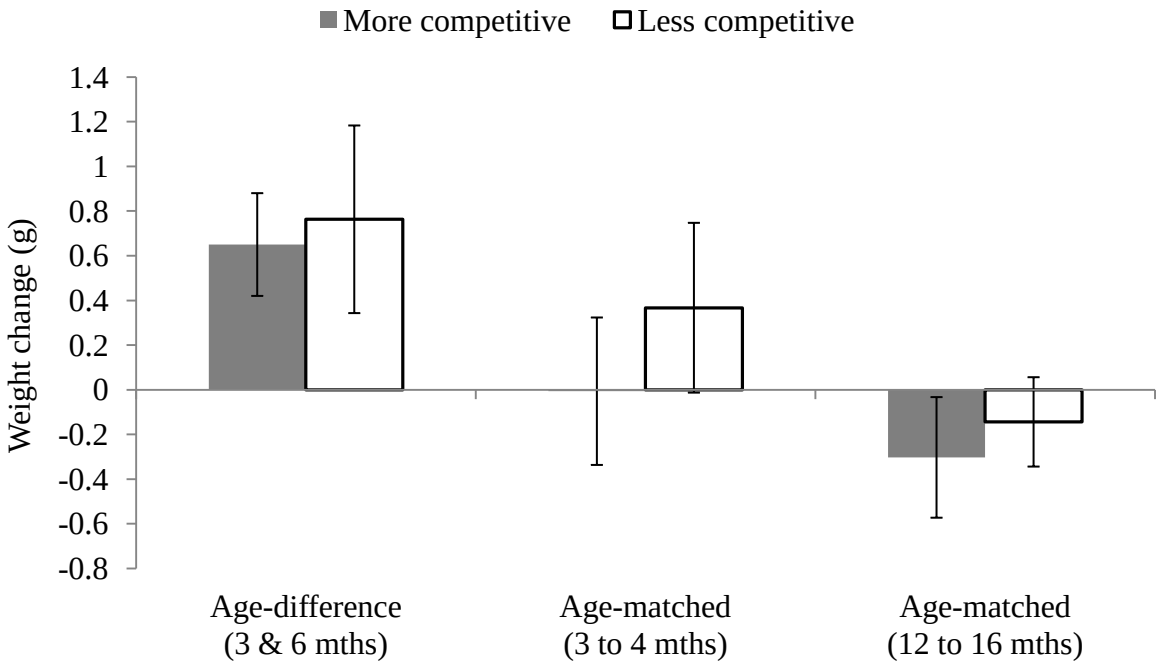


Figure 4.2 – Weight change following competitive female interaction tests for each age category (mean ± se).

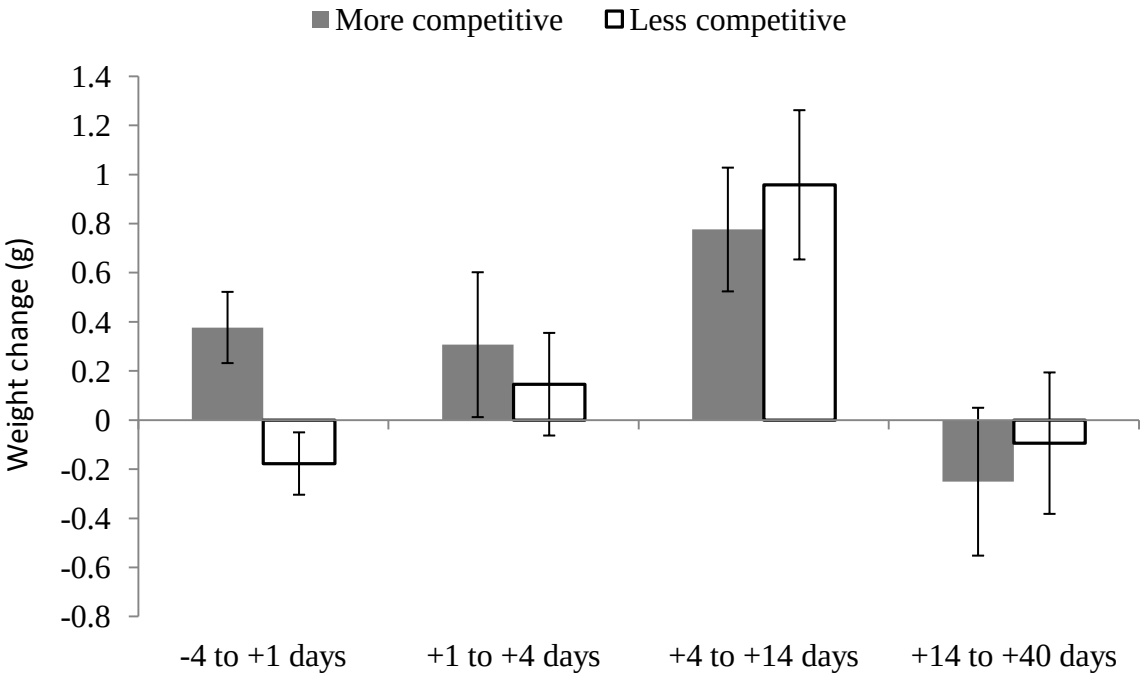


Figure 4.3 – Weight change for age-matched females at 12 to 16 months.
Females were weighed 4 days prior to the interaction day immediately before limited access to a sexually mature male (-4 days) and immediately before competitive female interaction (+1 day). Further weight measurements were taken 4 days after competitive female interaction (+4 days) and 14 days after female interaction (+14 days), immediately before limited access to a mature male. A final weight measurement was taken 40 days following female interaction (+40 days).

4.4.3 Urinary testosterone

When females from all treatment groups were used in statistical analysis, absolute measures of urinary testosterone significantly increased following competitive interaction for more competitive females ($Z = -3.721$, $n = 54$, $p < 0.001$), and there was a non-significant trend for increased testosterone for less competitive females ($Z = -1.877$, $n = 49$, $p = 0.061$). There was no correlation between female age and testosterone change for more competitive females ($r^s = -0.195$, $n = 50$, $p = 0.174$), however there was a non-significant negative correlation between age and testosterone change for less competitive females, suggesting that younger females tended to show greater increases in testosterone following competitive interaction ($r^s = -0.273$, $n = 49$, $p = 0.058$). There was no correlation between competitive score and testosterone change for either more competitive or less competitive females ($r^s = 0.062$, $n = 50$, $p = 0.671$; $r^s = 0.126$, $n = 49$, $p = 0.390$ respectively).

When treatment groups were analysed separately, testosterone significantly increased for more competitive females when they were housed with a different aged partner ($Z = -2.947$, $n = 16$, $p = 0.003$) and with a same aged partner at 3 to 4 months of age ($Z = -2.900$, $n = 13$, $p = 0.004$) (Figure 4.4). There was however no difference in testosterone from pre to post competitive stages for more competitive females housed with a same aged partner at approximately 12 to 16 months of age ($Z = -1.303$, $n = 21$, $p = 0.192$; Figure 4.4). Although there was a non-significant trend for increased testosterone in less competitive females overall, no significant differences were detected when age groups were analysed separately (age-difference $Z = -1.647$, $n = 16$, $p = 0.100$; aged-matched 3 to 4 months $Z = -1.433$, $n = 13$, $p = 0.152$; aged-matched 12 to 16 months $Z = -0.224$, $n = 20$, $p = 0.823$; Figure 4.5).

Females paired with a different aged female or aged-matched at 3 to 4 months were reproductively experienced, whereas females aged-matched at 12 to 16 months were reproductively inexperienced (although they met sexually mature males in between pre and post sampling periods). Therefore it was important to examine the effects of reproductive experience on testosterone change, particularly due to the significant differences detected between age groups. However Wilcoxon tests revealed no significant effects of reproductive experience on testosterone change in more competitive ($Z = -1.229$, $n = 50$, $p = 0.219$) or less competitive females ($Z = -1.363$, $n = 49$, $p = 0.173$).

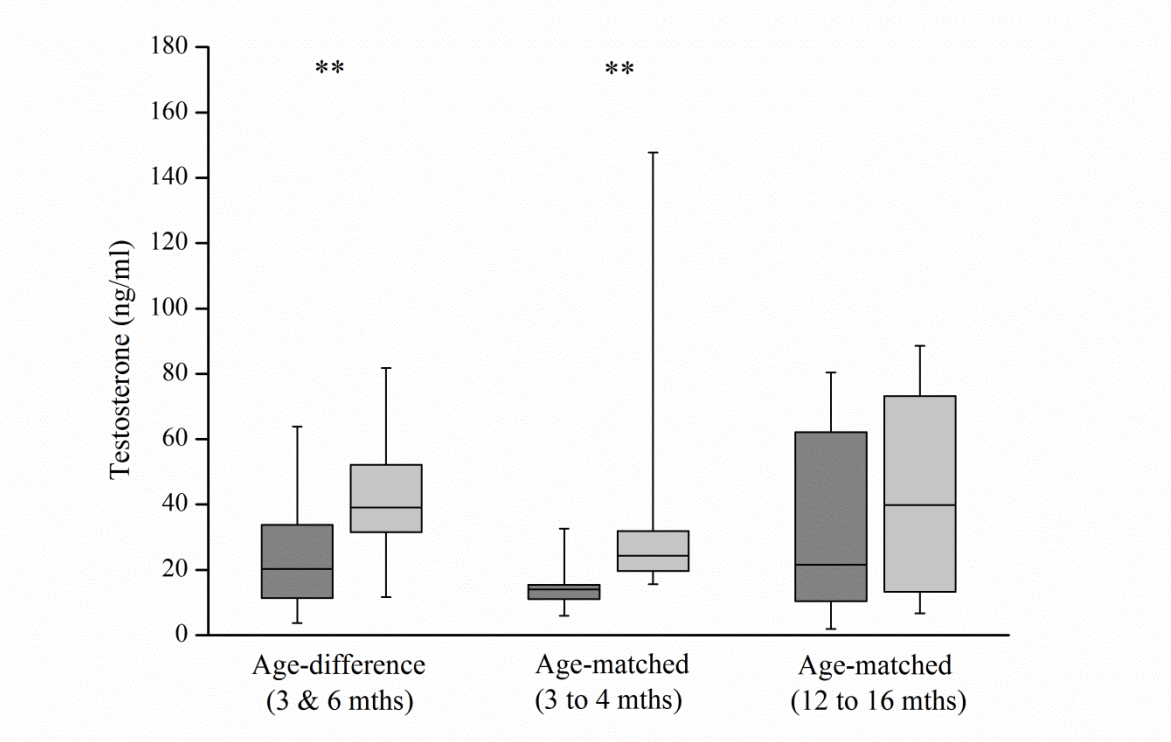


Figure 4.4 – Unadjusted urinary testosterone before (dark grey) and after (light grey) competitive female interaction for more competitive females ($p < 0.01$).**

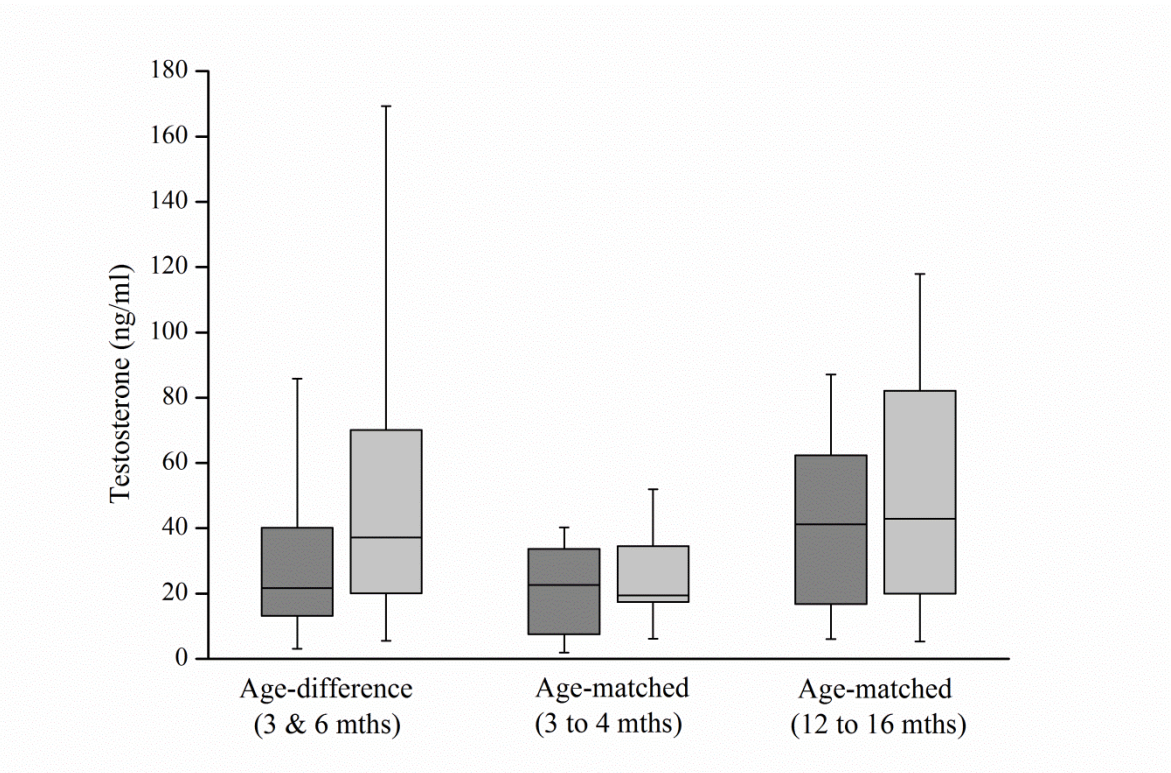


Figure 4.5 – Unadjusted urinary testosterone before (dark grey) and after (light grey) competitive female interaction for less competitive females.

4.4.4 Urinary protein

When females from all treatment groups were used in statistical analysis, urinary protein was found to significantly increase following competitive female interaction, both for more competitive females ($Z = -2.734$, $n = 54$, $p = 0.006$), and less competitive females ($Z = -2.067$, $n = 53$, $p = 0.039$). There was no correlation between female age and protein change for more competitive females ($r^s = -0.204$, $n = 54$, $p = 0.140$), however there was a significant negative correlation between age and protein change for less competitive females, suggesting that younger females had greater increases in protein following competitive interaction ($r^s = -0.323$, $n = 53$, $p = 0.018$). There was no correlation between competitive score and protein change for either more or less competitive females ($r^s = 0.037$, $n = 54$, $p = 0.788$; $r^s = 0.005$, $n = 53$, $p = 0.972$ respectively). There was no significant effect of reproductive experience on urinary protein for more competitive ($Z = -1.111$, $n = 54$, $p = 0.267$) or less competitive females ($Z = -1.083$, $n = 53$, $p = 0.279$).

When treatment groups were analysed separately the increase in protein was significant for more competitive females when they were housed with a different aged partner ($Z = -2.275$, $n = 16$, $p = 0.023$), and with a same aged partner at 3 to 4 months of age ($Z = -2.613$, $n = 15$, $p = 0.009$) (Figure 4.6). There was however no difference in protein levels from pre to post competitive stages for more competitive females housed with a same aged partner at 12 to 16 months of age ($Z = -0.365$, $n = 23$, $p = 0.715$; Figure 4.6). When less competitive females were examined, there were no significant differences in urinary protein when females were housed with a different aged partner ($Z = -1.396$, $n = 16$, $p = 0.163$) or with a same aged partner at 12 to 16 months ($Z = -0.146$, $n = 22$, $p = 0.884$) (Figure 4.7). There was however a significant increase in urinary protein following competitive interaction for less competitive females housed with a same aged partner at 3 to 4 months of age ($Z = -2.158$, $n = 15$, $p = 0.031$; Figure 4.7).

When analysing the data it appeared that older females (12 to 16 months) had higher levels of urinary protein compared to females in the both the young age-matched and the age-difference groups (see Figures 4.6 and 4.7). The results of a univariate GLM confirmed this ($F_{[2,104]} = 22.571$, $p < 0.001$), however there was no significant difference in urinary protein output between females in the 3 to 4 month age-matched group and age-difference group ($p = 1.000$).

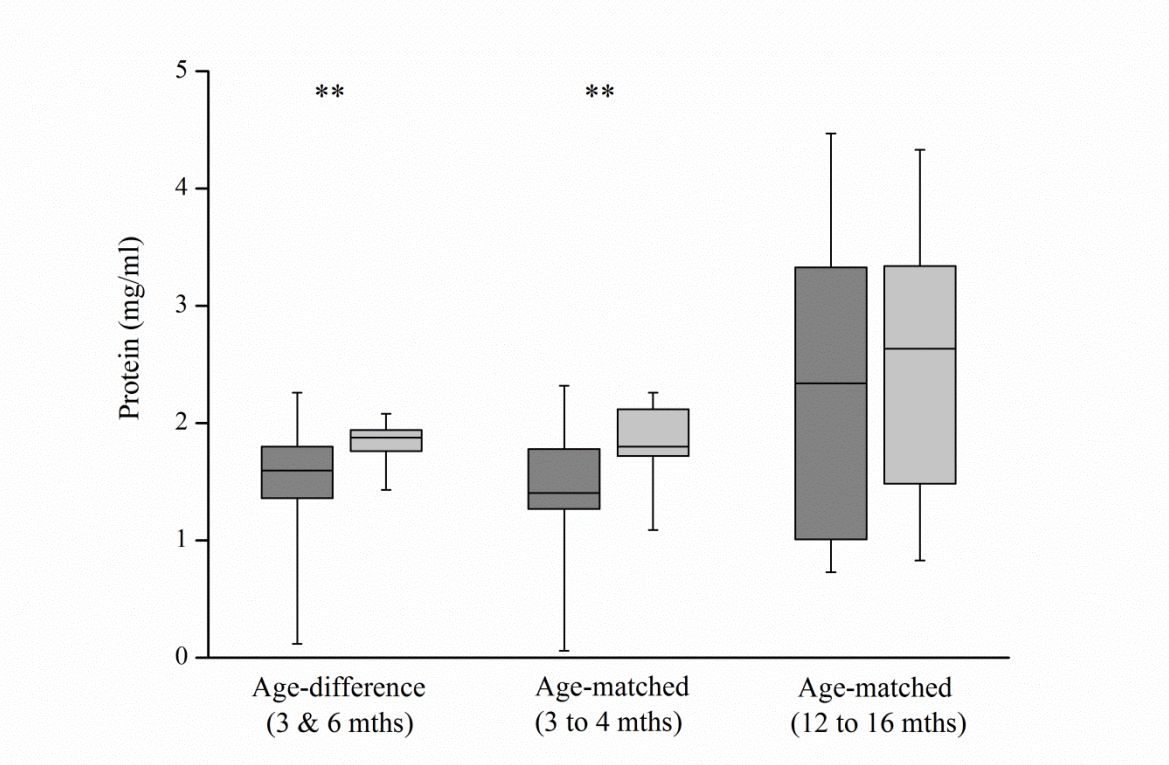


Figure 4.6 – Unadjusted urinary protein before (dark grey) and after (light grey) competitive female interaction for more competitive females (p < 0.01).**

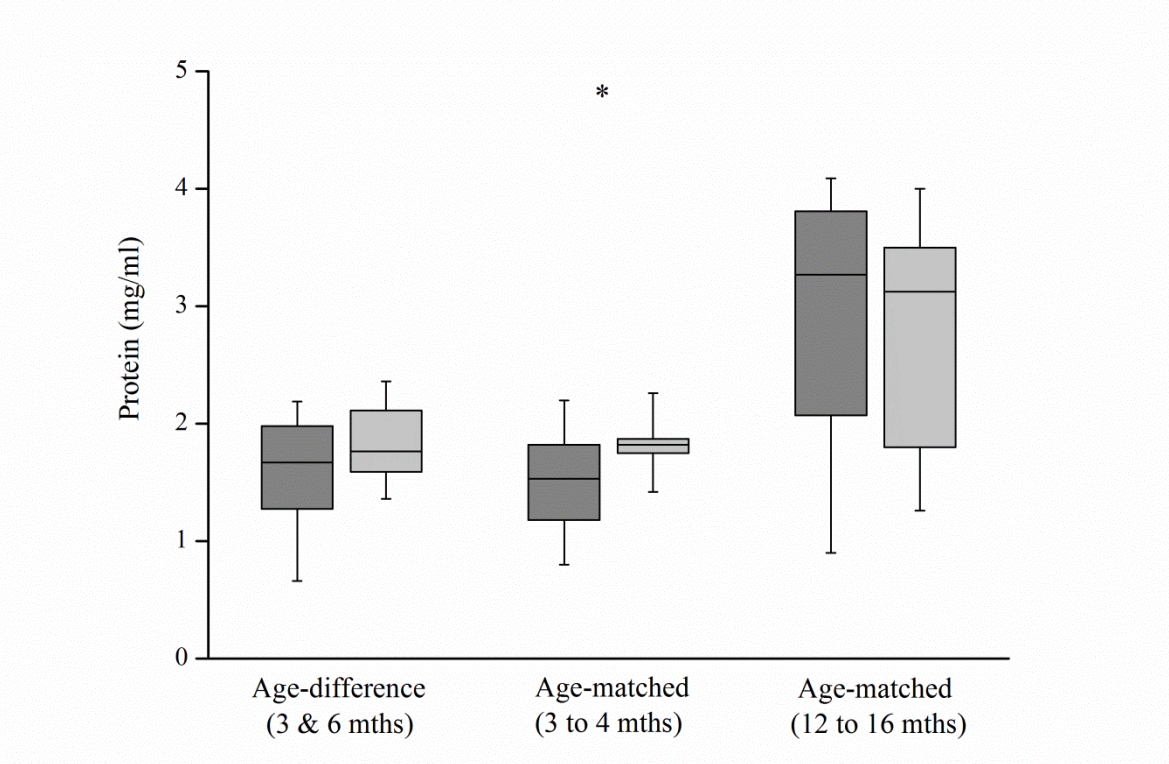


Figure 4.7 – Unadjusted urinary protein before (dark grey) and after (light grey) competitive female interaction for less competitive females (* p < 0.05).

4.4.5 How does female age influence physiological change when females are housed with a different aged social partner?

Although aged-matched female pairs could be directly compared using the previous statistical analysis, there was no comparison of how body mass, testosterone and protein changed for older (6 to 7 months) and younger (3 to 4 months) females in the age-difference group. This group was therefore analysed separately.

Changes in body mass were not significantly different between older and younger females ($t_{[15]}$ = 1.532, p = 0.146), as both older and younger females tended to increase in weight following competitive female interaction (older $t_{[15]}$ = -2.379, p = 0.031; younger $t_{[15]}$ = -1.860, p = 0.083). Urinary testosterone changes were also not significantly different between older or younger females (Z = -0.454, n = 15, p = 0.650), as both significantly increased urinary testosterone following competitive interaction (older Z = -2.172, n = 16, p = 0.030; younger Z = -2.272, n = 16, p = 0.023). There was however a non-significant trend for greater changes in urinary protein in younger females ($t_{[15]}$ = 1.927, p = 0.073), as younger females had significantly higher urinary protein following competitive interaction ($t_{[15]}$ = -3.700, p = 0.002), but older females showed no significant change ($t_{[15]}$ = -1.032, p = 0.318).

4.4.6 Does scent marking behaviour change following competitive experience?

Scent marking behaviour in the presence of a sexually mature male was investigated in an experiment with older aged-matched female pairs (12 to 16 months of age). The frequency and average size of scent marks deposited were compared 4 days prior to female competitive interaction and 14 days following interaction. Example scent mark patterns for more and less competitive females are illustrated in Figure 4.10.

Scent mark frequency in the presence of a male was significantly decreased following competitive experience for more competitive females ($F_{[1,19]}$ = 17.143, p = 0.001; Figure 4.8), however there were no significant relationship between the changes in scent mark frequency and changes in urinary testosterone, body mass, urinary protein, or with competitive score or female age (p > 0.050). Scent mark frequency was also significantly decreased following competitive experience for less competitive females ($F_{[1,20]}$ = 7.504, p

= 0.013; Figure 4.8), however there were no significant relationship between the changes in scent mark frequency and female age, changes in body mass, urinary testosterone, urinary protein, or with competitive score ($p > 0.050$).

The average size of scent marks deposited by more competitive females was also significantly reduced following competitive interaction ($F_{[1,20]} = 11.647$, $p = 0.003$; Figure 4.9), but there were no significant relationships between changes in scent mark size and changes in urinary testosterone, urinary protein, female competitive score, age in days or changes in body mass ($p > 0.050$). The average size of scent marks deposited by less competitive females were not significantly different following competitive interaction ($F_{[1,19]} = 0.106$, $p = 0.748$; Figure 4.9). There were also no significant relationships between changes in average scent mark size and changes in urinary testosterone or urinary protein ($p > 0.050$). There were however significant negative relationships between changes in scent mark size and changes in body mass ($F_{[1,19]} = 12.531$, $p = 0.002$), and also with competitive score ($F_{[1,19]} = 9.072$, $p = 0.007$). This suggests that the average size of scent marks deposited by less competitive females increased when their competitive score was relatively low and body mass was reduced. There was also a significant positive relationship with female age ($F_{[1,19]} = 10.401$, $p = 0.004$) suggesting that relatively older females deposited larger scent marks.

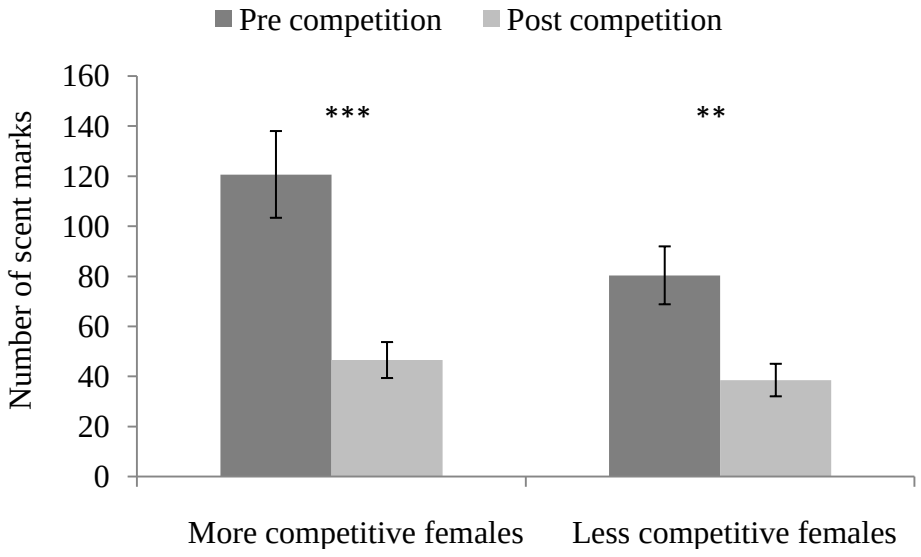


Figure 4.8 – Mean (± se) frequency of scent marks deposited by females in the presence of a male 4 days prior to competitive interaction and 14 days following (p < 0.01; *** p < 0.001).**

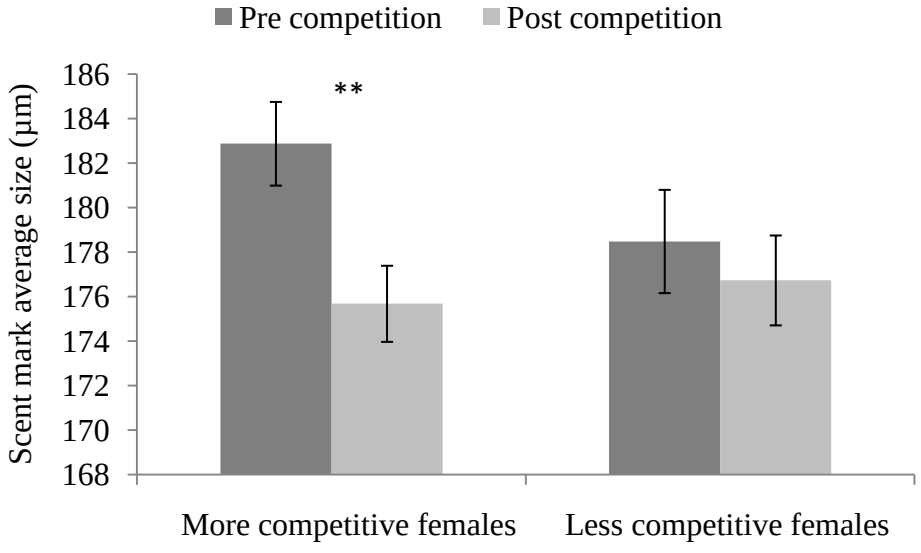


Figure 4.9 – Mean (± se) size of scent marks deposited by females in the presence of a male 4 days prior to competitive interaction and 14 days following (p < 0.01).**

a) More competitive female

Pre competition

Post competition

b) Less competitive female

Pre competition

Post competition

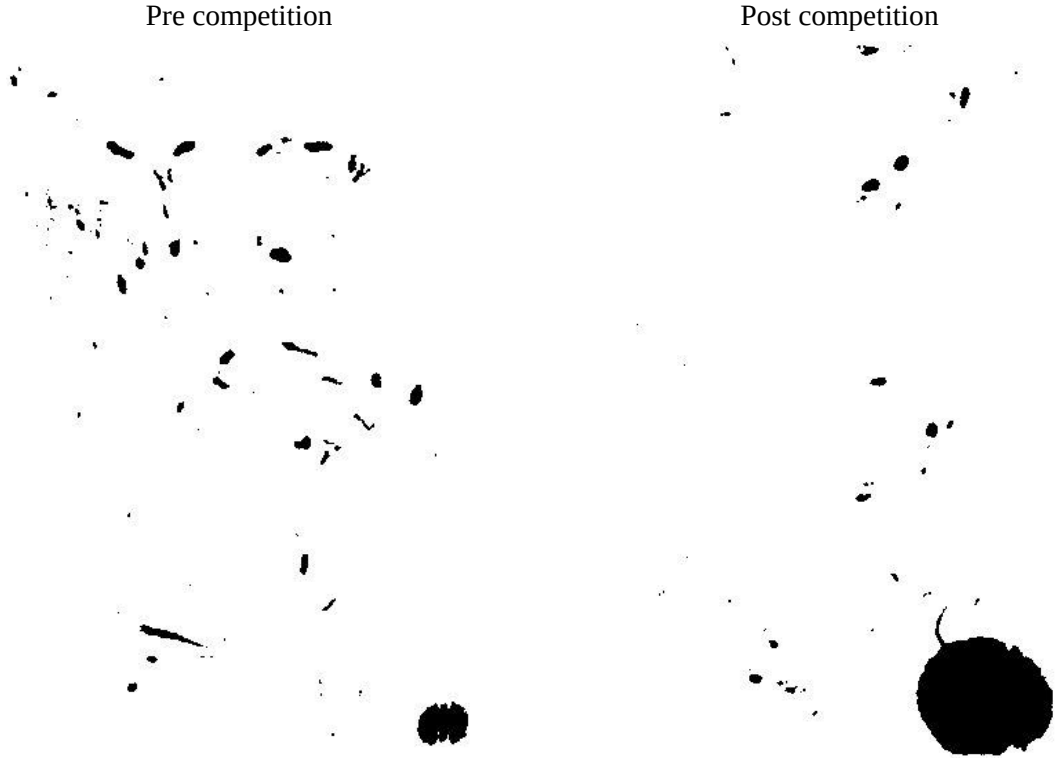


Figure 4.10 – Example scent marks deposited by more competitive (a) and less competitive (b) females, before and after competitive female interaction.

Scent marks were deposited by females in the presence of males (where males could make limited contact with females through a wire mesh barrier to the left of each image). In this example the most competitive female deposited scent marks along the edge of the mesh barrier and around the rest of the arena, but did not pool urine following competitive interaction (a). The least competitive female deposited fewer marks by the barrier and pooled urine in the corner on the opposite side of the cage to the male (b).

4.4.7 Dissection measurements

Adrenal glands of more competitive females in the older age-matched treatment group were not significantly lighter or smaller at dissection than adrenal glands of less competitive females when corrected for body mass (weight U = 235.000, n = 45, p = 0.683; length U = 235.000, n = 45, p = 0.523). Average gland sizes are reported in Table 4.1 and pictured in Figure 4.11.

When the adrenal glands of competitive females were compared to similarly aged stock animals that had previously been housed with sisters, both more competitive and less competitive females had significantly larger adrenal glands compared to stock animals (U = 60.000, n = 35, p = 0.010; U = 30.500, n = 34, p = 0.001 respectively). There were however no differences in adrenal weights between stock females and more competitive or less competitive females (U = 95.000, n = 35, p = 0.433; U = 71.000, n = 34, p = 0.113 respectively; Table 4.2).

Clitoral glands of more competitive females were not significantly heavier or larger at dissection than clitoral glands of less competitive females when corrected for body mass (weight U = 73.000, n = 25, p = 0.555; length U = 64.500, n = 25, p = 0.747). Gland sizes are reported in Table 4.1 and pictured in Figure 4.11.

Clitoral glands could only be detected in two of the twelve stock animals and therefore a statistical test could not be performed to detect differences in size or weight of clitoral glands taken from competitive females in this experiment. However the limited data collected suggested that clitoral glands could be approximately twice as heavy in more competitive and less competitive females compared to stock animals when correcting for body mass (Table 4.2).

Table 4.2 – Summary of adrenal and clitoral gland length (mm)/body mass and gland weight/body mass for more and less competitive females (mean ± se).

	Adrenal length (mm)	Adrenal weight (mg)	Clitoral length (mm)	Clitoral weight (mg)
More competitive	0.100 (± 0.008)	4.600 (± 1.710)	0.112 (± 0.013)	1.300 (± 0.430)
Less competitive	0.109 (± 0.007)	3.200 (± 0.610)	0.099 (± 0.013)	2.500 (± 0.790)

Table 4.3 – Summary of adrenal and clitoral gland length (mm)/body mass and gland weight/body mass for competitively housed females and females housed with sisters from general stock (mean ± se).

	Adrenal length (mm)	Adrenal weight (mg)	Clitoral length (mm)	Clitoral weight (mg)
Stock	0.066 (± 0.006)	1.500 (± 0.400)	0.056 (± 0.002)	0.600 (± 0.430)
Competitive	0.105 (± 0.005)	3.900 (± 0.920)	0.107 (± 0.009)	1.800 (± 0.440)

a) adrenal glands

b) clitoral glands

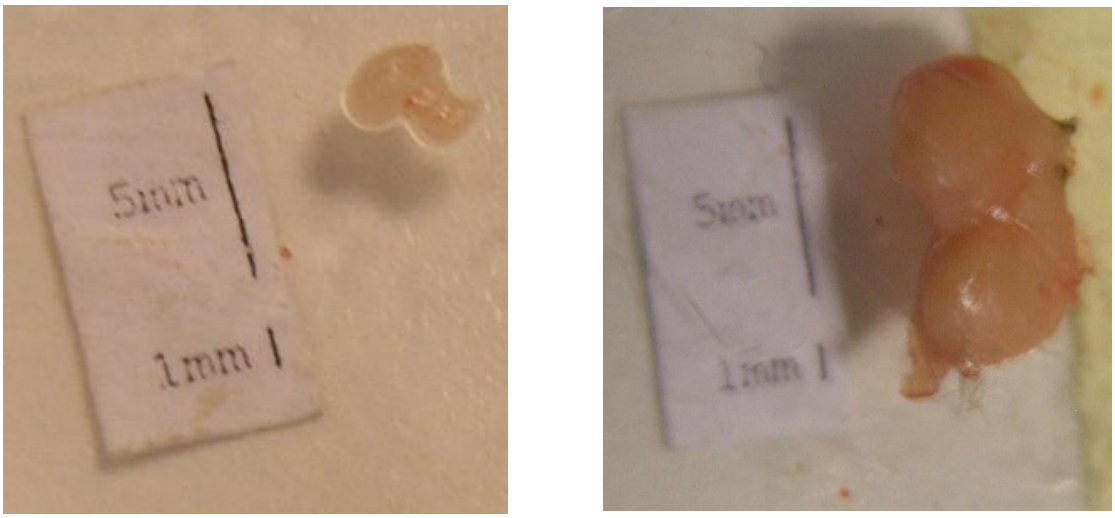


Figure 4.11 – Photographs of adrenal (a) and clitoral (b) glands taken during post mortem examination

4.4.8 Oestrus cycle monitoring

Oestrus cycle length was monitored in an experiment with older age-matched female pairs (aged 12 to 16 months) before and after female competitive interaction. There was however no significant difference in cycle length from pre to post competitive interaction for more competitive females (pre 3.3 $\pm$ 0.2 days, post 3.0 $\pm$ 0.2 days; Z = -1.633, n = 11, p = 0.102) or less competitive females (pre 3.2 $\pm$ 0.1 days, post 3.4 $\pm$ 0.3 days; Z = -0.962, n = 11, p = 0.336). Oestrus synchronicity between competitive female pairs was also not found to correlate with competitive score asymmetry following competitive interaction (r_s = -0.093, n = 21, p = 0.689).

4.4.9 Changes in relative intensity of MUP peaks following competitive experience

Changes in total MUP peak intensities following competitive interaction were not significantly different between more and less competitive females when paired with an aged-matched partner at 12 to 16 months ($t_{[20]}$ = -1.730, n = 20, p = 0.099).

Although the general pattern of MUP peaks expressed by individual females did not change between pre and post competitive interaction periods, the relative intensity of specific MUP peaks did appear to fluctuate for many females. For example, there was an increase in the relative intensity of MUPs expressed at mass 18,681 Da following competitive female interaction for both more and less competitive females (0.14 and 0.22 respectively). MUPs expressed at mass 18,725 Da however tended to increase for less competitive females by an average relative intensity of 0.19, whereas there was little change in expression for more competitive females (average decrease of 0.01). Some MUP peaks were only expressed by 1 or 2 individuals and therefore comparisons of relative intensity change could not be made between more and less competitive females in this experiment.

The majority of females (47%) consistently expressed peaks of highest relative intensity at mass 18,708 Da, while 21% consistently expressed highest peaks at 18,693 Da, 9% at mass 18,725 Da, 5% at 18,648 Da and 5% at 18,725 Da. Three females altered the mass of their highest peak by reducing relative intensity by at least 0.1 following competitive interaction, and increasing intensity of another peak. Two less competitive females reduced the relative intensity of MUPs at mass 18,693 Da following interaction and increased

intensity of MUPs expressed at masses 18,708 Da or 18,725 Da. Conversely a more competitive female reduced the relative intensity of MUPs at mass 18,648 Da, and increased expression of MUPs at mass 18,693 Da following competitive interaction.

4.5 Discussion

Overall it appears that competitive female interaction results in significant physiological changes in wild house mice. The age of female social partners appeared to be highly influential for the strength of the physiological response in more and less competitive females. In addition, younger less competitive females were more likely to show greater increases in urinary protein and testosterone.

4.5.1 Effects of competition on body mass

In the short term there was some evidence that body mass increased for more competitive females, but only when they were paired with an aged-matched female at three months, or an different aged partner of three or six months of age. Competitive score was influential in this change; the higher the score (i.e. the more aggressive a female was), the more her weight increased. All females appeared to increase in muscle mass four days after competitive interaction (as shown by an increase in urinary creatinine levels), despite the lack of an overall increase in body mass. If females are more active during the period of social rank formation, then it is possible that the increase in muscle mass correlates with a decrease in body fat. Therefore body composition could temporarily change during this time without effecting overall body mass (Randall *et al.*, 2000; Schmidt-Nielsen, 1997). It is possible that the more competitive female monopolised access to the food source, which may have resulted in weight gain, however there were no observable changes to body condition for any females in this experiment. Over a longer sampling period, body mass significantly increased between one and two weeks following competitive interaction for older aged-matched females, so it possible that any changes in body mass could be detected for younger females over the same period. Weight fluctuated throughout the 40 day weighing period and although final weight was not significantly different from baseline mass measured prior to female interaction tests for more competitive females, it significantly increased for less competitive females. In male house mice, body weight is thought to be influential in dominance relationships between unrelated individuals (Van Zegeren, 1980), and in the previous chapter I have shown that heavier females are more likely to be successful in competitive conditions with other females. Body mass only temporarily changed for more competitive females, and increased overall for less competitive females, but this may have been the result of differences in general behaviour. Less competitive females spend significantly less time in activity during competitive trials

(see Chapter 3) and may have increased in weight due to accumulation of fat. More competitive females may struggle to maintain competitive rank if their nest partners become heavier, although this would also require high levels of energetic investment and stamina from less competitive individuals.

4.5.2 Effects of competition on urinary protein, MUP production and scent marking behaviour

Following competitive female interaction, significant increases in urinary protein were detected for both more and less competitive females, particularly when females were paired with an age-matched female at three months old. When females were paired with a social partner of a different age, younger less competitive females were more likely to show greater increases in urinary protein, but more competitive females significantly increase investment in protein. Although older, reproductively inexperienced females (over 12 months) showed no difference in their urinary protein or relative intensity of MUP peaks following competitive interaction, they had higher urinary protein levels. A small number of less competitive females reduced the expression of particular MUPs at masses 18,693 Da, while a more competitive female increased expression of MUP at this mass. Both 18,693 Da and 18,694 Da are produced by both sexes and found in many laboratory strains of mice (Mudge *et al.*, 2008; S. A. Roberts, personal communication) and therefore it is difficult to determine why this would happen. There is currently very little known about urinary signalling in female mice, and particularly the role of MUP signalling (J. L. Hurst, personal communication). MUP concentration is thought to show some variation throughout the oestrus cycle (S. A. Roberts, personal communication; Stopka *et al.*, 2007) but there has been no attempt to examine the effects of competitive experience on MUP signalling in female mice. It would therefore be interesting to look at these findings in more detail as MUPs expression could be important in signalling competitive ability or reproductive status. When investigating the important characteristics for competitive potential in Chapter 2, I discovered that urinary protein levels were relatively higher in more competitive females, but there was no effect of age on competitive score. Changes in the relative intensity of MUP peaks was not examined for younger females aged between three and six months. As relatively younger females showed a significant increase in total urinary protein, it is possible that the investment in MUP signalling could also have changed in response to competition.

Olfaction is the dominant method of communication between house mice (Johnson, 1973) and high concentrations of protein are deposited when scent marks are deposited (Humphries *et al.*, 1999). By increasing investment in proteins, females may be able to increase the quality of the signals in their scent marks, which can provide information on social and health status (Hurst & Beynon, 2004). Consistent with dominant male scent marking behaviour, more competitive females in this experiment deposited smaller scent marks after competitive experience, which may have changed in response to social status. As a general trend, relatively younger females (i.e. 12 months old), with higher competitive scores and increased body mass were more likely to deposit smaller marks, suggesting a potential trade off in scent marking behaviour with age. Scent mark frequency however was reduced on average for all females following competitive interaction. If females scent mark to advertise their breeding status (Hurst, 1990d), then it is surprising that the frequency of marks was reduced when they had experienced living with a competitive social partner. Females were in oestrus at the time of testing and therefore reproductive cycle stage cannot explain the reduction in mark frequency. If females were energetically compromised as a result of the competitive environment they were housed in, then they may have been less motivated to deposit scent marks due to the associated energetic cost (see below). Urinary protein and testosterone levels did not appear to significantly influence scent mark frequency following competitive interaction, which is inconsistent with general trends observed in dominant male scent marking behaviour (Malone *et al.*, 2005). Although the function of the clitoral gland is not well known, gland secretions are thought to vary throughout the oestrus cycle and therefore could be used to signal breeding status in conjunction with urine components (Achiraman & Archunan, 2006; Achiraman *et al.*, 2011a; Achiraman *et al.*, 2011b; Jemiolo *et al.*, 1994). However secretions such as farnesenes are known to be costly to produce in male mice (Malone *et al.*, 2005). Therefore if the combination of urinary components and gland secretions result in a particularly strong and long-lasting signal, then it may not pay to deposit a large quantity of scent marks, particularly in females due to their investment in reproduction and lactation.

In this experiment older females living with competitive social partners had enlarged clitoral glands compared to similar aged females that had lived with siblings for the majority of their lives. The preputial gland of dominant male mice can be twice the size of subordinate male glands (Novotny *et al.*, 1990) and therefore it is reasonable to suggest

that clitoral glands may have increased in response to the competitive environment that experimental females had experienced. It would be particularly interesting to examine scent marking behaviour and the size and weight of clitoral glands from younger females to determine if there are links between scent mark frequency and gland size, independent of female age.

4.5.3 Effects of competition on urinary testosterone

Both the age of the female and that of her partner appeared to be important in predicting the strength of changes to urinary testosterone levels. Younger, less competitive females showed greater testosterone increases following competition; but overall, less competitive females did not significantly increase urinary testosterone. More competitive females increased testosterone output when paired with a different aged partner or a same aged partner at three months of age, but older females housed with an aged-matched partner did not. As older females had limited contact with a male under experimental conditions between pre and post sampling periods, this may have affected their response. However, by maintaining levels of testosterone, older reproductively inexperienced females may be more prepared to aggressively compete to increase their potential to breed, particularly as fertility could be compromised with age (Berry & Bronson, 1992). Clitoral glands may be influenced by testosterone, or at least an individual's sensitivity to the presence of androgens (Racey & Skinner, 1979), which may explain the difference in clitoral gland size for competitively experienced females (see above). However, the costs associated with high levels of testosterone can be detrimental to female fecundity, as demonstrated in bird species such as the zebra finch (*Taeniopygia guttata*) (Rutkowska *et al.*, 2005). Elevated testosterone can also increase energetic demand, reduce fat stores and increase the risk of injury due to increasing amounts of aggressive behaviour (Wingfield *et al.*, 2001). Individuals with high levels of testosterone may take more risks, increasing stress levels and production of glucocorticoids as part of the flight or fight response (Creel *et al.*, 1996), which also can affect reproductive success (Takahata *et al.*, 2006). Therefore there may need to be a trade off in production of testosterone.

4.5.4 Stress responses to competitive environments

When older age-matched female pairs were examined at the time of their death (13 to 17 months old), it appeared that their adrenal glands were significantly larger compared to

stock animals of the same age. This suggests that females living with a competitive social partner may have chronic stress responses, resulting in increases in size of the adrenal gland (e.g. Christian & Davis, 1966; Haller *et al.*, 1999). Enlargement of the adrenal glands occurs as a consequence of higher adrenal cortex activity in response to stress (Barnett, 2009). Although glucocorticoids were not measured in this experiment, the evidence of enlarged adrenal glands could suggest that corticosterone output may have also increased in response to competitive female interaction in wild house mice. Adrenal glands are not only involved in the production of glucocorticoids as part of the stress response, but are also involved in the production of sex steroids such as testosterone (Sapolsky, 2002). As testosterone was consistently produced by older competitive females in this experiment, it may provide another explanation of why adrenal glands were significantly larger in experimental females.

Interestingly, the oestrus cycle did not appear to be affected by competitive female interaction. Cycle length did not significantly change and synchronicity of cycles between social partners was not correlated with the amount of aggression females performed at introduction. This suggests that despite evidence of stress, paired females are able to sustain reproductive cycles. However there is still potential for female competition in the presence of a sexually mature male; by interrupting mating attempts (e.g. Sommer & Rajpurohit, 1989), and/or monopolising access to breeding males (e.g. Doran-Sheeny *et al.*, 2009). Females can also affect reproductive success through harassment during pregnancy (e.g. Packer *et al.*, 1995). It is therefore interesting to consider if female competition affects mating attempts in house mice, and if mate choice is affected by the physiological changes observed in this experiment.

4.6 Conclusion

The results of this experiment revealed that physiological changes do occur in response to female competition in wild house mice. Despite a temporary increase in body mass between one and two weeks following competitive interaction, competitive females appeared to maintain relatively similar weights, but less competitive females gained a significant amount of weight over 40 days post interaction, which could potentially compromise social rank. Urinary protein and testosterone was generally increased in more competitive females, but there was no change in the relative intensity of MUP peaks expressed by older females. Further tests could examine the investment of MUP signalling

in younger females, due to the increases observed in urinary protein. Scent marking behaviour by more competitive females appeared to be consistent with the patterns shown by dominant male mice, however there was a significant decrease in scent mark frequency which was unexpected. It is possible that the cost of scent marking was too high if females were energetically restricted as a result of the stress response. As older competitive females in this experiment were found to have enlarged clitoral glands it is possible that the excreted components of scent marks were adjusted in response to competition and therefore fewer marks were required to advertise breeding status (and/or competitive ability). This however is speculative and future tests should further examine the role of the clitoral gland in female scent marking behaviour. It would also be interesting to investigate the physiological impact of the stress response on wild house mice to determine if adrenal glands are enlarged due to increased production of glucocorticoids, or if it is a response to an increase in testosterone.

Competition between communally breeding females has a physiological impact on individuals which may influence future reproductive success. Surges in circulating testosterone could negatively affect reproductive success in the communal nest and therefore it is important for females to choose social partners carefully, in order to reduce the effect that the stress response may have. Females that can readily adapt to competitive conditions may therefore have an advantage over less competitive females in communal breeding conditions, however there is still potential for females to compete for reproductive opportunities with breeding males. Physiological changes and differences in scent mark behaviour could therefore influence mate choice, particularly if females communicate social and breeding status through their scent marks.

Chapter 5 Effects of female competitive ability on male mate choice

5.1 Chapter overview

The study of mate choice in mammals has predominantly focused on female choice of males, however there is increasing evidence that males may show a preference for some females, resulting in increased reproductive success. As I have previously shown that competition occurs between unfamiliar female social partners in wild house mice, with both body mass and urinary protein levels important in predicting competitiveness, I investigated whether competition can also influence male mate preference. Using a number of different experimental assays I found that males showed a mating preference for less competitive females, although they did not discriminate between competitive female partners on the basis of scent alone. Prior to female competitive interaction males appeared to spend more time investigating the odour of females with relatively lower levels of urinary protein (which may indicate lower competitive potential in females), but this was not apparent shortly after female competitive interaction. During preference tests for females, males were more likely to spend longer in the cage of females that they interacted more frequently with, and prior to female interaction males also spent more time in the cages of females that retreated more frequently from them. This suggests that males may show preference for less competitive females, possibly as they are less likely to be aggressive on first encounter and more competitive females may suffer from fertility costs. Female competition however may continue to play a role throughout the gestation period, potentially affecting reproductive success of both females in the communal nest.

5.2 Introduction

Mate choice occurs when individuals show a preference for mating with a particular category of partner, irrespective of mating success (Clutton-Brock & McAuliffe, 2009). A large number of species exhibit female mate choice, with dominant males being preferred due to fitness benefits they can provide, such as high-quality genetic traits to pass onto young, access to quality breeding sites and defence from predators (Bateman, 1948; Clutton Brock & Parker, 1992; Halliday, 1983; Trivers, 1972). Male mate choice has long been thought to be less common due to the differences between the sexes in reproductive

investment in gametes (Darwin, 1871; Trivers, 1972), but may evolve when females are encountered simultaneously (Barry & Kokko, 2010). Therefore the availability of mates and capability of a male to access breeding females is important (Edward & Chapman, 2011). On encountering a sexually mature female, males can choose to reject or accept courting females or they may themselves court particular females they are attracted to (Tudor & Morris, 2009). There have been a number of studies investigating the occurrence of male preference between females, particularly in terms of female size. For example, larger females are preferred in fruit flies (*Drosophilia melanogaster*) (Byrne & Rice, 2006), mottled sculpins (*Cottus bairdi*) (Downhower & Brown, 1981), fresh water shrimp (*Astellus spp.*) (Manning, 1975) and wood frogs (*Rana sylvatica*) (Berven, 1981), with all authors suggesting this was due to benefits on fecundity. Males may also select mates using other female characteristics such as mating history in vole species (*Microtus ochrogaster, Microtus montanus*) (Ferguson *et al.*, 1986), or moulting timing in hermit crabs (*Pagurus nigrofascia*) (Suzuki *et al.*, 2012).

5.2.1 Mate choice in house mice

Like many other species, female house mice exhibit mate choice, choosing mating partners on the basis of dominance status, as this predicts their ability to maintain and defend the territory in which the female will rear her offspring (Drickamer *et al.*, 2000; Malone *et al.*, 2001). The genetic components of competitive traits may then be passed onto their offspring, potentially increasing their reproductive success in later life (Thom *et al.*, 2008a). It was typically assumed that male house mice show little, if any discrimination among potential female partners, however in an experimental study Gowaty *et al* (2003) found that when male house mice were mated with preferred females, they sired more offspring and their sons were more likely to become dominant. There is also evidence that male house mice may discriminate between females on the basis of body mass as Costello *et al* (2009) found that males spent longer investigating females that were a similar weight to themselves. Male house mice tend to be larger than females (Berry, 1981), suggesting that males prefer relatively larger females, which as I have previously discussed, can potentially influence the number of offspring produced in a litter.

Communally nesting female house mice usually express synchronised oestrus cycles and mate with several different males over a breeding period as a result of visiting numerous male territories (Hurst, 1987; Mackintosh, 1981). Unfamiliar females are likely to be

encountered during this time, increasing competition between females for mating opportunities. Synchronised oestrus between female group members is thought to be a strategy against infanticidal behaviour (Palanza *et al.*, 1996), however it also results in a short period of receptivity for many females who then could compete for mating opportunities with available males (Petrie, 1983). Harassment and interruption of mating attempts by rival females may occur to enable competitive females to monopolise reproduction during synchronised oestrus, which is particularly important in species where sperm may be limited (see Emlen & Oring, 1977; Stockley, 2004; Stockley & Bro-Jørgensen, 2011; Stockley & Preston, 2004). It is therefore essential that females are able to maximise their chances of mating during the relatively short period of oestrus, perhaps mediated through competitive behaviour and/or scent communication.

5.2.2 Scent communication for mate assessment in house mice

Male house mice extensively scent mark their territory and countermark any marks deposited by intruders; a trait used by females in mate assessment (Rich & Hurst, 1998). Female urine contains high levels of protein and other components which may be important in signalling competitive ability (Arakawa *et al.*, 2008; Drickamer, 2001; Harvey *et al.*, 1989). In addition, high levels of androgens associated with aggression and masculinisation (such as testosterone) have been suggested to constrain fertility (Packer *et al.*, 1995), which may therefore negatively influence male assessment of potential mates. This information could be used by males to assess female quality, however signals could change with competitive experience (see Chapter 4). Reproductive experience may also affect scent marking behaviour, as non-breeding females are thought to rarely deposit scent marks (Hurst, 1990c). But if competitive ability is also communicated through urine then scent marking behaviour may change in response to competitive experience.

Major urinary proteins (MUPs) have an important role in female attraction of mates (Roberts *et al.*, 2010) and individual recognition in house mice (Cheetham *et al.*, 2007). MUPs are excreted in high quantities in urine and saliva and MUP peak profiles are thought to be stable throughout life (Beynon & Hurst, 2004). House mice have been shown to use MUPs to avoid inbreeding (Sherborne *et al.*, 2007), and in a recent study, females spent more time in proximity to sexually mature males that had dissimilar MUP profiles to themselves (Holmes, 2012). It is therefore important to examine the degree of similarity of MUP peaks shared between females and males, as this could influence male preference.

5.2.3 Experimental aims

In this thesis I have shown that female house mice perform competitive behaviour when they first meet an unfamiliar and unrelated female. As competitive ability may predict female reproductive success, it is also possible that males may select female mates on the basis of competitive behaviour and physiological characteristics. Males may also prefer to sire offspring with more competitive females if they can defend offspring more effectively (Petrie, 1983), however competitive females may also respond aggressively towards males, or be more likely to perform infanticide against their offspring (Palanza & Parmigiani, 1994). The level of intra-sexual competition perceived by females may also influence their behaviour, for example females may respond more aggressively towards a female rival when a sexually mature male is encountered. Male preference may therefore change in response to the changes in female behaviour and/or scent communication following competitive social housing.

In this experiment I investigate whether males show a preference for females of differing competitive ability prior to and following the establishment of female dominance relationships. To achieve this I measure the amount of time males investigate females and their scent, both before and after female dominance relationships have been established, as competitive signalling and other potential cues of female attractiveness may change as a result. In addition I quantify competitive interactions between female social partners and a breeding male, as well as mating behaviour, to investigate if females compete for mating opportunities and look for evidence of female mating interference. If chemical signals in female urine indicate their competitive ability, the amount of time males spend investigating female urine could also be affected.

5.3 Methods

5.3.1 Animals

Female mice used in this experiment were reproductively inexperienced and aged approximately 12 to 16 months old (n = 48). In line with previous findings, females were expected to show some competitive behaviour at the time of introduction to an unfamiliar, unrelated female social partner (see Chapter 3). To test for male preference within female pairs, sexually mature males were used in this experiment (n = 94), each aged between 7 to 18 months and were reproductively inexperienced. Individuals were classified as unrelated if they did not share more than one great-grandparent. Prior to testing all animals were housed in conditions described in Chapter 2, Section 2.1. Females were fitted with a RFID tag and their tail or fur marked for visual identification using the methods described in Chapter 2, Section 2.2. Two females died over the time period of this experiment and therefore samples sizes were reduced from 24 pairs to 22 pairs for the final tests.

5.3.2 Experimental procedure

5.3.2.1 Competitive female interaction

At least one week prior to testing (Table 5.3) females were moved in groups of 2 to 3 familiar sisters into a clean MB1 cage containing the enrichment items as previously described. This was to ensure that females were not housed in a way that may disrupt the oestrus cycle, as groups of 4 or more females can become reproductively suppressed (Van Der Lee & Boot, 1955; Van Der Lee & Boot, 1956). Four days prior to competitive interaction, females were weighed and allocated to a test pair by matching body mass between females (0 to 2 g difference). On the test day females were tested to ensure they were in oestrus (see Chapter 2, Section 2.5) and introduced to an unfamiliar and unrelated female social partner in a test arena as described in Chapter 2, Section 2.6. At the end of the 30 minute test, females were transferred to a specially adapted MB1 cage, bisected laterally with a Perspex barrier (45 x 13 cm), with a section cut out along the middle covered on either side with aluminium wire mesh (mesh spacing 0.5 cm). Females within each pair were housed either side of the barrier. This allowed females to have limited tactile and visual contact while maintaining olfactory contact with their new social partner. Cages also had a specially adapted wire lid to enable water bottles and food pellets to be

placed on either side of the mesh barrier. The bottom of each cage was lined with substrate and contained paper wool nesting material as described in Chapter 2, Section 2.1. Females were housed in their divided cages for 5 days, during which time they were introduced to one another on 2 more occasions in the test arena with 40 to 56 hours between each test. To ensure all females were tested during the red light phase, only 12 pairs were introduced on each test day with tests conducted from 09:00 to 18:00 hours. To control for order effects of testing females throughout the day, 3 blocks of 4 pairs were rotated during each of the 3 trials (Table 5.1). If females were excessively aggressive (see ethical rule in Chapter 2, Section 2.7) then the test was stopped and the animals were returned to their divided cage. At the end of the third test, females were transferred to a standard MB1 cage and housed with the social partner they had been introduced to. These cages contained all of the standard housing enrichment as described in Chapter 2, Section 2.1. DVD recordings of behaviour during the 3 tests were watched blind to the identity of the mice and the frequency of competitive behaviours (attack, chase and fight) and submissive behaviours (retreat, submissive posture) were recorded (Chapter 2, Table 2.1).

Table 5.1 – Interaction trial order for experimental pairs

	Test 1	**Test 2**	**Test 3**
Test order on experimental days	Pairs 1 to 4	Pairs 5-8	Pairs 9-12
	Pairs 5 to 8	Pairs 9-12	Pairs 1-4
	Pairs 9 to 12	Pairs 1-4	Pairs 5-8

5.3.2.2 Male preference tests

Subject males were tested for their preference of females before and after females had established a competitive relationship. Female urine samples were also used to determine whether males may show a preference based on female odour cues.

a. Male preference for females

Two tests were conducted during this experiment; the first test 4 days prior to competitive female interaction and the second test 2 weeks after female competitive interaction (see Section 5.3.4 for a schedule). Different males were used in tests 1 and 2 to ensure that preference was not based on memory from a previous test. Approximately 5 hours before testing, urine was collected from females for odour preference tests (see Section 5.3.2.2b) using the recovery method and immediately frozen at -22^{o}C (see Chapter 2, Section 2.3). A swab test was performed to establish the stage of oestrus and ensure both females within a test pair were at the same stage (see Chapter 2, Section 2.5). Females were then weighed and returned to their home cages for approximately 3 to 4 hours prior to testing. To test male preference between females within a pair (n = 24), a series of 3 interconnected MB1 cages were used (Figure 5.1). The central cage was used as a 'neutral' cage with no female scent and was used as the start point for the experiment by placing the male inside. Each end cage was bisected laterally with a Perspex barrier (45 x 13 cm), with a section cut out along the middle covered on either side with aluminium wire mesh (mesh spacing 0.5 cm). Females were placed individually in each of the end cages, behind the wire mesh. This enabled olfactory, acoustic and visual contact between males and females, as well as limited tactile contact. The base of each end cage was lined with Benchkote to measure scent marking behaviour by both male and females, but no other items were placed inside the cages to maximise vision of movement of the mice on the overhead cameras. The cages were connected by clear Perspex tunnels (33 length x 5 cm diameter) to allow the male to move between each of the 3 cages, and up to the mesh barrier in each of the end cages. Clear Perspex perforated lids were also used in each of the 3 cages to enhance visibility of subjects when filming behaviour from above the apparatus. Night vision cameras (Panasonic CCTV camera WV-BP330/B with TV lens WV-LZ6215 1:1.6 8x zoom 5-40mm) were suspended from brackets above the apparatus and trials were recorded remotely to DVD (Panasonic video monitor WV-BM1410 and Panasonic DVD/HDD recorder DMR-EX769) in an adjacent room. Due to the short duration of the test food and

water was not placed inside the test apparatus, but following the test the subjects were immediately transferred to their home cages where food and water was available *ad. libitum*.

Males were habituated to the test environment for 30 minutes by placing them in an empty MB1 cage with a clear Perspex perforated lid to replicate the test environment. Females were placed in the end test cages and left for 30 minutes to habituate to the test environment. Trials lasted for 60 minutes, commencing once observations of the male entering both end cages had occurred. At the end of the trial all subjects were returned to their home cages. Benchkote was removed from the 2 end cages and stored in sealed plastic bags for analysis at a later date. Perspex tubes and lids were thoroughly cleaned in hot soapy water, rinsed under cold running water to remove any soap residue and allowed to air dry. Cages were autoclaved as standard laboratory practice. DVDs were watched blind to the identity of females. Duration of time and number of visits made by males to the end cages containing females were recorded, as well as the number of interactions between males and females through the mesh barrier. The frequency that females approached, followed or retreated from a male at the barrier was also recorded (see Table 5.2).

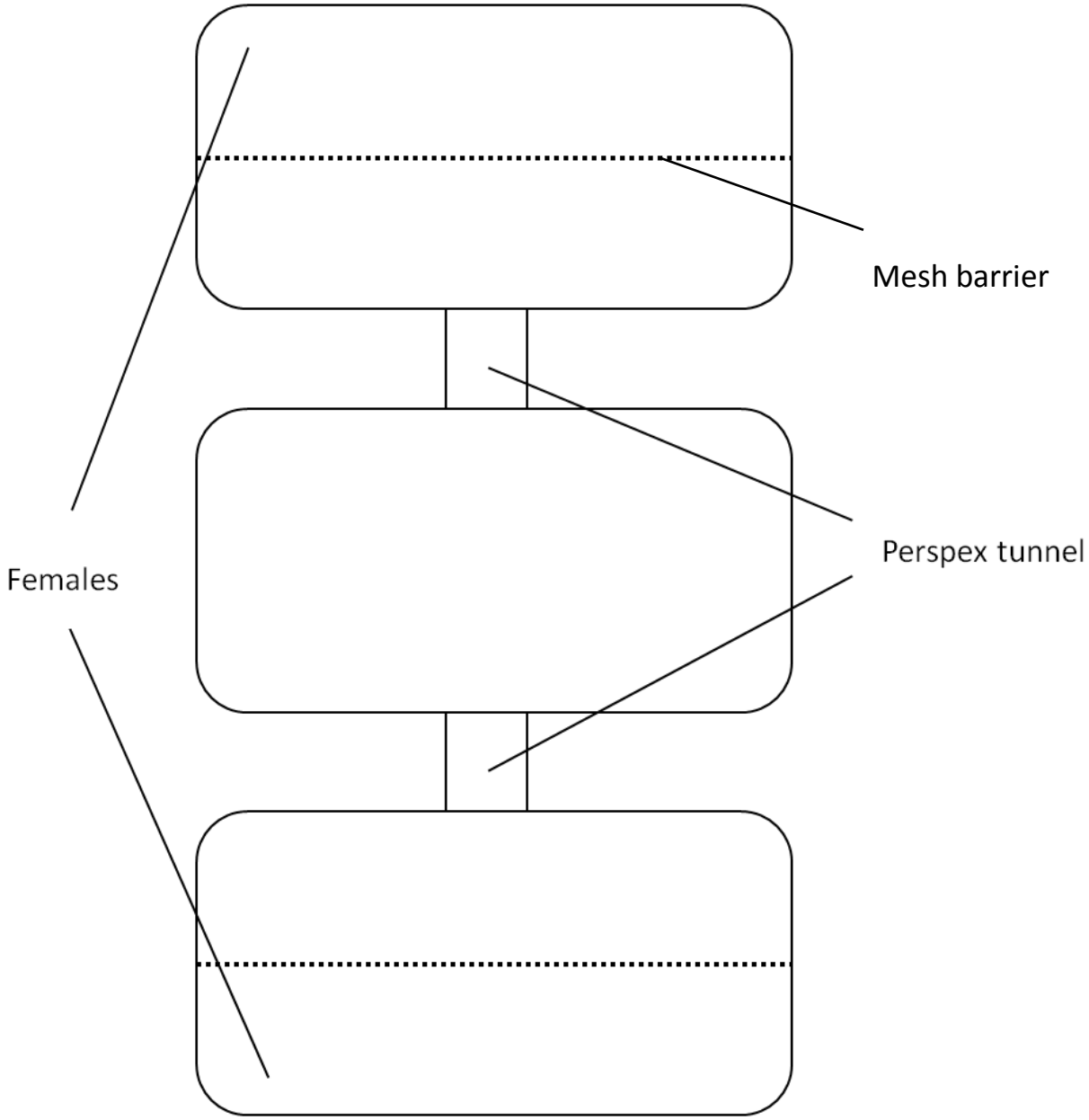


Figure 5.1 – Three cage test arena used in male preference tests.
Three MB1 cages were connected by tunnels, with end cages bisected laterally by Perspex tunnels to enable the male to move from the central cage to the end cages to interact with both females through the wire mesh.

Table 5.2 – Descriptions of female and male behaviour during experimental tests.
Behaviour descriptions have been modified from Oortmerssen (1971).

	Behaviour	Description
Male preference for female tests	**Approach**	Females move towards the mesh divider to within half a body length with nose pointed in the direction of the male.
	Follow	Walking behind or slightly behind but parallel to another individual, keeping to the same path as the leading individual.
	Retreat	Following an interaction or approach, rapid locomotion away from another individual, moving at an increased speed from an average walking pace.
	Interact	Individuals in close contact between the mesh barrier of the cage, either sniffing face/body of the other individual or using front paws to make contact.
Mating trials	**Mating mounts**	Males place back legs on the ground and front legs and body resting on the females back, usually with intromission movements.

b. Male preference for female odour

Pilot test

To determine if males showed a preference for female odour in the test arena I conducted a series of pilot tests with 22 males. Males were presented with odour from an unrelated and unfamiliar female and a related, familiar female, with the expectation that males would spend more time investigating and/or in proximity to unrelated, unfamiliar odour (see Hurst, 2009). Trials were conducted in clean laminated medium-density (MDF) arenas (70 x 60 x 55 cm) with 2 Perspex tiles (15 x 15 x 0.5 cm) placed at opposite ends of the arena approximately 10 cm from the edge of the wall of the arena. Tiles were covered in Benchkote to measure scent marking behaviour by the males. Stimulus urine (10 µl) was streaked onto each tile within a 5 cm diameter circle, drawn in pencil on the Benchkote in the centre of each tile. One tile was streaked with 10 µl of urine from a familiar sister, and 1 tile streaked with 10 µl of urine collected from a female unfamiliar and unrelated to the male subject. Urine was given 10 minutes to dry before testing. Males were given 30 minutes to habituate to an empty test arena prior to testing. Related and unrelated odours were presented in a randomised but balanced position within the arena to control for any potential side bias. Trials lasted for 15 minutes and male behaviour towards the 2 scents was recorded remotely to DVD using a camera mounted on a tripod at the base of the arena (as described in Chapter 2, Section 2.6). DVDs were watched blind to the position of each scent using a recorder programme written by R. J. Beynon. The amount of time males spent within the circle of each tile was recorded as a measure of proximity to odour, and the amount of time males spent sniffing the urine streaks was recorded as a measure of investigation. Males were recorded as sniffing female odour if they were standing still with their nose in contact with the circled area containing female odour (sometimes with small movements of the head and nose during investigation). The frequency and average size of male scent marks deposited on the Benchkote covered tile was calculated using the methods described in Chapter 2, Section 2.11.

A Wilcoxon Signed Ranks test was used to determine if males spent more time in proximity to and/or sniffing urine from an unrelated female. Two males were removed from the analyses as they did not sniff both female urine marks during the test. Males spent significantly more time in proximity to unrelated female odour ($Z = -2.277$, $n = 20$, $p = 0.023$). There was also a non-significant trend for males to spend more time investigating

unrelated female odour (Z = -1.717, n = 20, p = 0.086). As the pilot study showed that males visit both odour cues and that the 15 minute trial period was sufficient to detect a difference in investigatory behaviour, this method was incorporated into the main experimental design as an additional measure of male preference.

Experimental test

Two tests were conducted during this experiment, using the methods described in the pilot test above; the first odour test was conducted using urine collected from females 4 days prior to competitive female interaction and the second test using urine collected 2 weeks after female interaction (see Section 5.4.3 for a schedule). The position of each female stimulus urine mark was randomised across the 2 tests but balanced. The same male was used in each of the 2 tests to determine if preference for females changed from test 1 to 2 (i.e. following competitive female interaction). To ensure that males did not show memory for female odour or position (which may have biased results) a 'rest' period of 21 days was left between testing, as males have been shown to remember the location of scent for up to 14 days in a similar test environment (Roberts *et al.*, 2012). Tests were balanced for order with 12 males tested with female odour following competitive interaction first and 12 males tested with odour from females before competitive interaction first. Urine used in this experiment had previously been stored at -22^{o}C which has previously been shown to be as effective as fresh stimulus urine (Ramm *et al.*, 2008). Once again the amount of time males spent within the circle of each tile was recorded as a measure of proximity to odour, and the amount of time males spent sniffing the urine streaks was recorded as a measure of investigation. The frequency and average size of male scent marks deposited on the Benchkote covered tile was calculated using the methods described in Chapter 2, Section 2.11.

5.3.3 Mating outcomes following competitive experience

Eight to 10 weeks following competitive female interaction, female pairs were tested in a semi-naturalistic test arena with a sexually mature male to determine if mating behaviour matched male preference (if shown) and to determine if female competition resulted in interference of mating behaviour. Mating trials were conducted in 2 blocks of 12 pairs, with 4 female pairs tested each week for 3 weeks; pairs 1 to 4 and 13 to 16 were tested 8

weeks after competitive female interaction, pairs 5 to 8 and 17 to 20 tested 9 weeks after interaction, and pairs 9 to 12 and 21 to 24 were tested 10 weeks after interaction.

Three days prior to the test females were weighed and provided with soiled bedding from an unfamiliar and unrelated male to stimulate oestrus (see Chapter 2, Section 2.4). Male subjects were also provided with soiled bedding from the cage of female pairs they were to encounter in the test to familiarise males with female odours. On the test day, females were weighed and transferred to melamine enclosures (116 x 58 x 80 cm) with their social partner in their MB1 home cage with the lid left partially open to allow free access to the enclosure and the home cage. Individuals had *ad. libitum* access to food and water from the food hopper in the cage lid. Enclosures contained 2 Perspex sheets (27 x 23 x 0.3 cm) and 6 concrete bricks (20 x 3 x 3 cm) to provide areas of cover. Females were left to habituate to the test arena for 60 minutes. Males were then introduced to the enclosure housed in their M3 home cages with the lid closed for 30 minutes to allow females to approach and investigate the male cage. Full olfactory, acoustic and visual communication could occur during this period, as well as limited tactile contact through the cage lid. After 30 minutes the male cage lid was left partially open to allow all subjects to freely interact. Night vision cameras were suspended from brackets above the enclosures and behaviour was recorded continuously to a HDD/DVD recorder in an adjacent room (as described in Chapter 2, Section 2.8). Observations could be made during the experiment to ensure that all individuals were interacting and that no excessive aggression occurred during the trials (see ethical note in Chapter 2, Section 2.7). Filming commenced at the time of male introduction for approximately 90 hours. Female latency to initially approach the caged male was recorded, as well as frequency of competitive and submissive behaviours between females and males, and between females for the duration of the experiment (Chapter 2, Table 2.1). In addition, the frequency of male mounting, and the identity of the approaching animal prior to mounting were also recorded (Table 5.2).

At the end of each mating trial, females were checked for the presence of reproductive plugs and any signs of injury to the face, body or fur. Females were urine sampled using the recovery method before being transferred to their MB1 home cage with their female social partner. Males were removed from the enclosures at the same time as females and also checked for signs of injury before being transferred to a clean M3 cage and returned to the stock room.

5.3.4 Experimental schedule

Two blocks of 12 pairs of females were tested over a 12 week period with male preference tests for females performed 4 days prior to and 2 weeks following female interaction. Mating trials were conducted 8 to 10 weeks following female interaction (Table 5.3).

Table 5.3 – Experimental schedule.

Week	Test	
0	Females re-housed and primed for testing	
1	Male preference for females (pre competition)	Male preference for female odour (pre competition)
2	♀-♀ Interaction (x3)	
3	Oestrus/Weight monitoring	
4	Male preference for females (post competition)	Male preference for female odour (post competition)
5	Oestrus/Weight monitoring	
6	Oestrus/Weight monitoring	
7	Oestrus/Weight monitoring	
8		
9		
10	Mating trials	
11	Mating trials	
12	Mating trials	

5.3.5 Data analysis

Male scent marks deposited during male preference tests were analysed using the methods described in Chapter 2, Section 2.11. Urine samples collected from all individuals were also analysed for MUP mass spectra as described in Chapter 2, Section 2.10 to determine if male preference or mating behaviour related to MUP peak profile similarity between test females and males.

Where data did not meet parametric assumptions, a log transformation was applied. When data could not be normalised by transformation, non-parametric statistics were used for analysis. All statistical tests were carried out using SPSS software v20.

To test male preference for females, 3 types of tests were conducted. Firstly Wilcoxon tests were used to test male preference for more or less competitive females and their odours (when competitive rank was allocated on the basis of competitive score) during the female and odour preference tests and the mating trials. Wilcoxon tests were also used to determine if female behaviour towards males changed following female competitive interaction. Univariate general linear models (GLM) were then conducted to test for relationships between the time males spent in proximity to females within each pair and the differences between females (i.e. differences in competitive score, body mass, anogenital distance and female behaviour during the trial). Differences were calculated by subtracting values for the female with the lowest competitive score away from the female with the highest competitive score. As females were tested in two groups, block effects were also investigated in these analyses, but were removed from the model if they were not significant. Non-significant covariates where also removed sequentially until the minimal model was achieved.

As the differences between females did not take into account the range of female competitive scores or the strength of male 'preference', a multivariate linear regression was used to establish which characteristics were most influential in the time males spent in proximity to stimulus females. Finally Spearman's rank correlation tests were used to examine if there was a relationship between male preference and MUP peak profile similarity between the subject male and each females. However, MUP data was not available for all males used in this experiment and therefore sample sizes used in the analysis are reported in Table 5.7.

5.4 Results

5.4.1 Male preference for females

To determine what effect (if any) female competition had on male preference, I measured the amount of time males spent in proximity to females, using competitive score to distinguish competitive rank of females. However, Wilcoxon tests revealed that there was no difference in the average amount of time males spent in proximity to more or less competitive females prior to female competitive interaction ($Z = -1.343$, $n = 24$, $p = 0.179$) or two weeks following female competitive interaction ($Z = -0.061$, $n = 23$, $p = 0.951$). There was also no difference in the frequency of scent marks that males deposited in response to more or less competitive females either prior to female interaction ($Z = -0.806$, $n = 24$, $p = 0.420$) or after interaction ($Z = -0.015$, $n = 23$, $p = 0.988$).

5.4.1.1 Female behaviour towards males

Prior to competitive interaction, females did not differ in the frequency of interactions with males ($Z = -0.129$, $n = 24$, $p = 0.898$), or in the frequency of approach ($Z = 0.000$, $n = 24$, $p = 1.000$), retreat ($Z = -0.383$, $n = 24$, $p = 0.701$) or follow ($Z = -1.372$, $n = 24$, $p = 0.170$). Following competitive interaction, more competitive females did not interact with males more frequently compared to less competitive females ($Z = -0.374$, $n = 23$, $p = 0.708$), and did not approach or follow males more frequently (approach $Z = -0.244$, $n = 23$, $p = 0.808$; follow $Z = -1.126$, $n = 23$, $p = 0.260$). Less competitive females did not retreat from males more frequently than more competitive females following competitive interaction ($Z = -0.716$, $n = 23$, $p = 0.474$).

As male preference for females was tested before and after competitive female interaction, I compared the frequency of behaviours performed by females between the two trials to determine if competitive experience influenced motivation to interact with the male. However, female behaviour towards males did not change following competitive female interaction (Table 5.4).

When allocating relative competitive ability of females I could not determine if the asymmetry of competition between female pairs influenced the strength of male preference. I therefore calculated the differences in competitive score between female pairs and the difference in time that males spent with each female. Using this data I then

conducted a univariate GLM to determine if the difference in competitive score predicted the difference in time spent in proximity to females. I also calculated the differences in female behaviour between pairs and added these to the model. Results of the GLM revealed that before competitive female interaction, males spent more time in proximity to females that retreated from them more frequently ($F_{[1,21]} = 7.766$, $p = 0.011$), and also with females that interacted more frequently with them ($F_{[1,21]} = 29.762$, $p < 0.001$) (N.B. females could retreat from a male approach without an interaction occurring and therefore each behaviour was tested independently). There were no significant relationships between time males spent with either female and differences in female approach, competitive score, body mass or anogenital distance ($p > 0.050$) and therefore these covariates were removed from the analysis. Two weeks after competitive interaction, males spent more time in proximity to females that interacted more frequently with them ($F_{[1,21]} = 7.334$, $p = 0.013$). Differences in female approach, retreat, competitive score, body mass and anogenital distance were not significantly related to time males spent with females ($p > 0.050$).

5.4.2 Male preference for female odour

When competitive rank was allocated to individual females, Wilcoxon tests revealed that there was no difference in the average amount of time males spent in proximity to, or investigating competitive or less competitive female odour prior to female competitive interaction (proximity $Z = -1.460$, $n = 23$, $p = 0.144$; sniff $Z = -0.791$, $n = 23$, $p = 0.429$) or following competitive interaction (proximity $Z = -0.373$, $n = 23$, $p = 0.709$; sniff $Z = -0.231$, $n = 23$, $p = 0.831$). There was a non-significant trend for males to deposit more scent marks on or in close proximity to more competitive female odour prior to female interaction ($Z = -1.765$, $n = 23$, $p = 0.078$), but there was no difference in scent marks deposited near more or less competitive female odour following competitive female interaction ($Z = -0.260$, $n = 23$, $p = 0.795$).

Table 5.4 – Summary of Wilcoxon results to determine if female behaviour towards males changed from pre to post competitive interaction (n = 23).

	More competitive females		Less competitive females	
Behaviour towards males	**Z**	**p**	**Z**	**p**
Approach	-0.015	0.988	-0.091	0.927
Retreat	-0.751	0.452	-0.934	0.350
Follow	-1.125	0.260	-0.712	0.477
Interact	-0.137	0.891	-0.213	0.831

Table 5.5 – Summary of Wilcoxon results to determine if male investigation of female odour or scent mark frequency differed from pre to post competitive female interaction (n = 23).

	More competitive females		Less competitive females	
Time in proximity to female odour	Z = -0.958	p = 0.338	Z = -1.095	p = 0.274
Time investigating female odour	Z = -0.211	p = 0.833	Z = -0.517	p = 0.605
Frequency of scent marks deposited	Z = -0.766	p = 0.444	Z = -1.009	p = 0.313

As the same male subjects were used to test male preference for female odour prior to and following female competitive interaction I investigated whether male preference was consistent across the two test periods. Males did not change the amount of time they spent in proximity to or investigating more or less competitive female odour during the two test periods. Males also deposited a similar number of scent marks when in proximity to or investigating more or less competitive female odour, both before and after female competitive interaction (Table 5.5).

Male investigation of female odour could have been affected by competitive signals that females produced either prior to or following competitive interaction. Therefore the differences in female urinary testosterone and urinary protein levels were used in a univariate general linear model to determine if they related to differences in time that males spent investigating female urine. As urinary creatinine levels did not significantly change between the two sampling points ($t_{[46]}$ = -1.254, p = 0.216), urinary protein and testosterone levels were corrected for urinary dilution by dividing by creatinine (see Chapter 2, Section 13).

Prior to competitive female interaction, males spent significantly longer in proximity to and investigating female odour with relatively lower urinary protein content (proximity $F_{[1,19]}$ = 5.988, p = 0.024; sniffing $F_{[1,19]}$ = 6.907, p = 0.017). Differences in urinary testosterone and competitive score between females did not significantly influence the time males spent in proximity to or investigating female odour (p > 0.050), and were therefore removed from the analysis. However there was no relationship between the relative differences in urinary protein between females and the difference in time males spent in proximity to or investigating female odour post interaction (proximity $F_{[1,17]}$ = 0.001, p = 0.970; sniffing $F_{[1,18]}$ = 0.538, p = 0.473). This could not be explained by changes in female urinary protein levels, as no significant differences were detected between the two sampling points ($t_{[21]}$ = 1.519, p = 0.144).

5.4.3 Mating outcomes following female competitive interaction (mating trials)

Males were observed mounting less competitive females more frequently than more competitive females (average mounts with less competitive females = 20.14 and with more competitive females 3.23; Z = -2.366, n = 22, p = 0.018). There were no differences in the

frequency of aggressive or submissive behaviours between more and less competitive females directed towards the breeding male (Table 5.6). There were also no differences in the frequency of aggressive or submissive behaviours between more and less competitive females directed towards each other, except that less competitive females approached more competitive female partners more frequently (average approach by less competitive females = 70.68 and by more competitive females = 59.09; Z = -2.063, n = 22, p = 0.039) (see Table 5.6). Of the females that were mated, males were more likely to initiate mounting bouts than females (average approach by males 7.32 $\pm$ 3.83; by females 4.36 $\pm$ 2.26; Z = -1.960, n = 8, p = 0.050).

In an isolated event, a more competitive female began 'mounting' the less competitive female, shortly after the male had mounted the less competitive female. This event lasted for approximately 2 hours, and during this time the more competitive female was observed mounting 44 times, with intervals between each observation lasting between 1 and 15 minutes. Over this time period, the more competitive female also chased away the male if he approached either female. Only 4 more competitive females were observed interrupting mating behaviour between males and less competitive females; this was achieved by approaching the pair and chasing the male when he broke away from mounting the less competitive female. There were no observed instances where less competitive females interrupted mounting between males and more competitive females.

5.4.4 Influence of female MUP peak profile sharing on male preference

As MUP peak sharing between males and females is likely to influence male preference, I investigated if there were any significant relationships between the differences in MUP peaks shared between females and males, and male preference over the series of tests conducted in this experiment. Overall no significant correlations were detected, except during the female preference test prior to competitive female interaction, where there was a non-significant trend for males to spend more time in proximity to females that shared fewer MUP peaks with them (Table 5.7).

Table 5.6 – Summary of Wilcoxon results to determine if there was a difference in behaviour performed by more and less competitive females towards each other and the breeding male in the mating trials (n = 22).

	Female behaviour towards male		Female behaviour towards female	
Approach	Z = -0.921	p = 0.357	Z = -2.063	p = 0.039*
Chase	Z = -1.047	p = 0.295	Z = -1.186	p = 0.236
Retreat	Z = -0.852	p = 0.394	Z = -0.280	p = 0.779
Attack	Z = -1.350	p = 0.177	Z = -0.970	p = 0.332
Fight	Z = -0.679	p = 0.497	Z = 0.000	p = 1.000

Table 5.7 – Results of Spearman's rank tests to examine correlations between the differences in MUP peak sharing between females and males, and male preference for females, their odour or preference during mating trials.

		MUP peak sharing between males and females		
		r_s	n	p
Difference in time in proximity to females	**Pre female competitive interaction**	-0.478	14	0.084
	Post female competitive interaction	-0.181	13	0.554
Difference in time spent investigating female odour	**Pre female competitive interaction**	-0.308	22	0.164
	Post female competitive interaction	-0.118	23	0.593
Difference in time spent in proximity to female odour	**Pre female competitive interaction**	-0.152	22	0.501
	Post female competitive interaction	-0.113	23	0.608
Difference in frequency of mounting behaviour	**Post female competitive interaction**	0.213	14	0.464

5.5 Discussion

In conditions where females compete for reproductive opportunities or resources, male mate preference could be influenced by female behaviour and/or cues of competitive ability (Edward & Chapman, 2011; Petrie, 1983). In this study, males showed a mating preference for less competitive females, but female competitive rank did not appear to influence either male preference for females or examination of female odour. As all encountered individuals in this experiment were unrelated, males may have simply investigated both females (or scents) at similar rates, in order to gain as much information on health and breeding status, and to maximise opportunities of breeding with as many receptive females as possible (Clutton Brock & Parker, 1992). However, cues of competitive ability prior to female interaction may have influenced male preference for odour, as males spent more time investigating odour with relatively low urinary protein levels prior to competitive female interaction.

In odour tests conducted prior to female interaction, males tended to deposit more scent marks on or around more competitive female odour, as they would when countermarking rival male scent cues (Hurst, 1990d). However, as there were no differences in urinary testosterone or protein levels of more or less competitive females, it is unlikely that males were using either cue when countermarking. It is also unlikely that males would have mistaken females for rival males as urine has been shown to contain signals of gender (Cheetham *et al.*, 2007). Female house mice also signal breeding status through urine marks which is used by males to distinguish between receptive females so this signal would also have been present during the tests (Hurst, 1990c; Hurst, 1990d). Before competitive female interaction urinary protein was important for male attraction, but not afterwards, despite no significant changes in urinary protein levels between tests. This suggests that male attraction could be affected by other signals present in the urine, not investigated in this experiment. All females were in oestrus at both sampling periods and therefore reproductive cycle stage could not explain the difference. However, body mass did significantly increase for both more and less competitive females during this time, which may have affected urinary components (Drickamer, 1995 and see Chapter 3), although protein and testosterone were corrected for dilution using urinary creatinine levels.

During male preference tests for females, males spent more time in proximity to females they interacted with, regardless of female competitive rank or experience. Prior to competitive interaction, males also preferred to spend time with females that retreated more frequently from encounters, and females that shared fewer MUP peaks with them. As related individuals are more likely to share MUP peaks this suggests that males may have preferred females that appeared to be less related to them (i.e. inbreeding avoidance Sherborne *et al.*, 2007), however this preference was not found after females had interacted with their competitive social partner. Although there were no significant differences in the frequency of retreats or interactions between tests, it is possible that females reduced the amount of time they spent in the area of the cage furthest from the male following the retreat, and spent more time in the area closest to the mesh barrier waiting for the male to approach. It is possible that competitive experience increased motivation to interact with males, in order to maximise potential opportunities to mate (Petrie, 1983), but males may have been less willing to respond if females appeared to be more aggressive in their 'courtship'.

During mating trials, males were more likely to mount less competitive females and males also initiated most of the mounting bouts, suggesting that the male was actively seeking mating opportunities; although female response would also be influential in the success of this attempt (Cunningham, 2003). There were a small number of instances where more competitive females interrupted mating attempts between the male and the less competitive female. This suggests that females are potentially able to manipulate mating success within their social group, similar to female competition for mates observed in red-winged blackbirds (*Agelaius phoeniceus*) (Lenington, 1980). In one extreme event, an aggressive female mounted her social partner after observing her mating with the breeding male, chasing away the breeding male if he approached her or her social partner. This isolated incident left the male unable to attempt to mate with either female for the remainder of the test period which would consequently affect reproductive success. It is possible that the male used in this experiment was perceived as a particularly high-quality male, increasing the intra-sexual competition for access to him (Petrie, 1983), however the more competitive female made no attempt to approach the male or investigate him and was more interested in aggressively pursuing the female. It would have been interesting to examine the subsequent stress response and reproductive success of the females that were affected by direct aggression or interruption during mating trials. (Due to the timing of dissections

in the experimental schedule it was not possible to identify reproductive scars in the uterine horns of these mice and therefore I was unable to predict reproductive potential.) However, these could be isolated incidences. In general, less competitive females were more likely to approach female social partners during the mating trials, which may have been a strategy to signal subordinate status in the presence of a male (Aureli & Smucny, 2000). Opportunities to disperse in natural conditions are limited by availability of nest sites, but if females experience relatively high levels of aggression from their social partner then they may be more likely to attempt to disperse to avoid the risk of further injury (Clutton-Brock & Lukas, 2012; Konig, 1994a; Stephens *et al.*, 2005).

The results of this experiment suggest that males may prefer less competitive females however it is not clear how females may manipulate this choice. The preference for more subordinate females could be explained due to potential levels of aggression the male may experience when approaching an unfamiliar female with a more 'dominant personality'. In addition, high levels of testosterone observed in more competitive females in Chapter 3 could have a negative effect on fertility levels (e.g. Packer *et al.*, 1995). If males can gain information on urinary testosterone levels when investigating the female through scent, he may prefer to mate with a female with relatively lower testosterone as a measure of fecundity. Whilst observations of mating do not necessarily imply successful reproduction, where subordinate females are mated first it is probable that they will also give birth first, giving their pups weight advantages over the second litter born in the communal nest. This would suggest that more competitive females suffer a significant reproductive disadvantage, which is surprising. There are however other opportunities for more competitive females to compete throughout gestation which could result in resorption of unborn foetuses in subordinate females (Huck *et al.*, 1988b), and there is substantial evidence of infanticidal behaviour by female house mice close to parturition, which would increase the number of available lactating females for the infanticidal female's young (Maestripieri, 1992; Manning *et al.*, 1995; Palanza *et al.*, 1996). Therefore more competitive females may allow their social partners to mate with breeding males, but direct aggression towards them to induce resorption. Alternatively they may allow less competitive females to give birth to their young before performing infanticide to reduce or eliminate the number of pups present in the nest that would compete with their own offspring when they are born; this has previously been observed in banded mongooses

(*Mungos mungo*) (Bell *et al.*, 2012) and meerkats (*Suricata suricatta*) (Clutton-Brock *et al.*, 1998).

5.6 Conclusion

Mate choice in wild house mice is generally to be performed by females who prefer more dominant males to sire their offspring, while males maximise reproductive success by mating with multiple females. Previous studies have shown that male house mice have higher reproductive success when they mate with a preferred female, but there has been no attempt to determine how female competition could influence male mate choice. In this experiment males appeared to preferentially mate with less competitive females, and they spent longer investigating female odour with relatively lower urinary protein. It is therefore possible that male mate preference could be influenced by female behaviour and/or competitive status. More competitive females may not be perceived as high quality mates (Packer *et al.*, 1995); alternatively more competitive females may allow their social partners to breed first to manipulate birth order, before performing infanticide to increase the ratio of lactating females to dependent offspring. The next chapter therefore investigates the effect of female competition on reproductive output and division of maternal care performed by female pairs.

Chapter 6 The effects of female competition on reproductive output and maternal care in house mice

6.1 Chapter overview

In the previous chapter I found that where female pairs were housed with a male in a semi-naturalistic environment, less competitive females were typically the first to mate. To investigate how female competition may influence reproductive output after mating, I investigated the reproductive output of females housed with a competitive social partner. Two groups of females were tested, one age-matched (aged three months) and one group with an age-difference of approximately three months (aged three and six months old), as age has previously been shown to affect the level of competition between paired females. Litter size, mass and sex ratio were measured under both solitary and communal conditions, as well as pup survival and weight gained from birth to weaning. Reproductive output was reduced in the communal nest compared to previous reproduction in a solitary nest, however reproductive output was increased when communal partners shared more MUP peaks. A higher frequency of more competitive females compared to less competitive females gave birth in the communal nest, however there was no difference in the total number of pups weaned by more and less competitive social partners. Survival rates were not significantly different between more and less competitive partners suggesting that both females suffered from pup mortality. Although there was no overall difference in latency to birth between more and less competitive females, females that gave birth first had fewer pups present in the nest on post natal day one compared to the second female. There was also a non-significant trend for first born offspring to gain more weight on average than pups born second. Time that females spent in proximity to communal offspring was not significantly different between more and less competitive females, even when one female was not lactating, but when the number of pups present in the nest was controlled for, there was a trend for more competitive females to reduce the amount of time they spent in proximity to pups in the communal nest. Finally there was evidence to suggest that females born to competitive females had higher average litter sizes, and that females born in competitive environments such as communal nests were more likely to have male biased litters. Together these results suggest that female competition results in a reduction in reproductive output in the communal nest, either through resorption or infanticidal behaviour, but there was little evidence for more competitive females to gain

reproductive advantages over less competitive social partners. However, competitive rank of dams and/or early experience could both be influential for the future reproductive success of their offspring.

6.2 Introduction

Communal breeding systems describe social groups with one or more breeding females rearing their combined young in a single nest and sharing offspring care (Sayler & Salmon, 1971). This system is traditionally thought to be more egalitarian than singular (or cooperative) breeding systems where one female monopolises reproduction (Solomon & French, 1997). Many species exhibit communal breeding, ranging from small species such as Goeldi's marmoset (*Callimico goeldii*) (Saltzman *et al.*, 2009), and the spiny mouse (*Acomys cahirinus*) (Hayes, 2000), to larger species such as banded mongooses (*Mungos mungo*) (Gilchrist, 2006), and spotted hyenas (*Crocuta crocuta*) (Frank, 1986). Studies of reproductive success in the latter two species have been of particular interest in recent years due to the presence of dominance hierarchies between females, which can enhance reproductive success through priority access to important resources such as food or high-quality mates (Bell *et al.*, 2012; Frank, 1986; Gilchrist, 2006). More dominant females are often larger and heavier than less competitive females and consequently are more likely to produce larger litters than smaller social partners (Hodge *et al.*, 2009). Birth order is also thought to be important for reproductive success in many communally breeding species. By giving birth first, offspring gain size and weight advantages over the subsequent litter born in the nest, enabling them to effectively compete against their littermates for access to lactating females (Hodge *et al.*, 2009).

Androgenisation has also been extensively studied in species such as the spotted hyena (*Crocuta crocuta*), as females are larger and more aggressive than males (Frank, 1986). In this species androgenised females gain advantages during competitive feeding, play major roles in defending clan members and also in protecting offspring from infanticidal individuals (Glickman *et al.*, 1998). Whilst the previous authors suggested that androgenised females in spotted hyenas suffered no deleterious effects on fertility, high levels of circulating androgens have been shown to have inhibitory effects on cycling and ovulation in other mammal species (Packer *et al.*, 1995). Polycystic ovaries in human females produce high levels of circulating androgens (namely testosterone), sometimes resulting in infertility (Franks, 1995). In laboratory rats, provision of androgens eliminates

cycling and ovulation (Feder, 1981), whereas in dogs and primate species, androgen provision delays the onset of puberty (Beach *et al.*, 1983; Goy & Resko, 1972). The presence of androgens can also affect maternal behaviour by impairing pup retrieval in rats (Bridges *et al.*, 1973) or maternal nest building in rabbits (Anderson *et al.*, 1970), suggesting that androgenisation could potentially be detrimental throughout the reproductive lifespan.

Although the costs and benefits of communal breeding can apply to all species living in this social system, mammalian species have an additional cost related to lactational demand, as each female is likely to provide milk for the combined offspring (Hayes, 2000; Konig, 2006; Packer *et al.*, 1992; Roulin, 2002). There is therefore huge potential for competition between communally breeding females. Competitive females may suppress reproduction of others to reduce competition for reproductive resources and to enable the competitive female to coerce suppressed females into caring for her offspring (Cant & Johnstone, 1999). Reproductive suppression can occur directly through harassment and aggression, or indirectly through signals of dominance status (Creel *et al.*, 1992; Wasser & Barash, 1983). In some rodent species, increasing group size can also result in reproductive suppression (Ma *et al.*, 1998; Van Der Lee & Boot, 1955; Van Der Lee & Boot, 1956). In this instance non-breeding females may queue for reproductive opportunities and inherit breeding positions in the future (e.g. Hodge *et al.*, 2008; Kokko *et al.*, 2002). Infanticidal behaviour may also occur in the communal nest, particularly if births occur a few days apart (Hager & Johnstone, 2004; Hodge *et al.*, 2011). Birth synchronisation has therefore been widely suggested as a strategy to protect against infanticidal behaviour (Agrell *et al.*, 1998; Ebensperger, 1998; Hager & Johnstone, 2004; Hrdy, 1979; Poikonen *et al.*, 2008).

6.2.1 Maternal effects in competitive environments

The success of individuals depends not only on the ability to survive and to successfully reproduce, but also to produce offspring that are more successful than the offspring of competitors (see Cunningham & Birkhead, 1998; Dawkins, 1989). This may be achieved through mating with high-quality individuals or through differential investment in reproduction (Cunningham & Birkhead, 1998; Russell & Lummaa, 2009). Maternal effects describe the condition when a mother's phenotype or environment can influence the phenotype of offspring (over the direct effect of transmitted genes) (Marshall & Uller, 2007). These effects can be anticipatory, meaning mothers may increase investment in

their offspring before birth (e.g. through increased growth in-utero) to provide them with competitive advantages in challenging environments. Mothers may increase the number of potential helpers in less competitive environments by increasing investment towards daughters, whereas in competitive or densely populated communal nests, females may adjust investment towards male offspring that would disperse from the natal area (Russell & Lummaa, 2009). Consequently, female offspring born into male biased litters are likely to be affected by high levels of androgens which may increase competitiveness (Gandelman *et al.*, 1977; Vom Saal, 1978), but potentially delay sexual maturity (McDermott *et al.*, 1978). By producing larger offspring, mothers can enhance their offspring's competitive ability over littermates (Hodge *et al.*, 2009). However, if maternal fitness is maximised by the quantity rather than quality of offspring she produces, then mothers may search for a new territory in which to breed, to enable her to produce more numerous young (Marshall & Uller, 2007).

6.2.2 Communal breeding in house mice

The adaptive benefits of communal breeding on lifetime reproductive success has been the focus of many studies in wild house mice (Hayes, 2000; Konig, 1994a; Manning *et al.*, 1995; Reimer & Petras, 1967; Sayler & Salmon, 1971). Communal breeding enables a small group of females to share the costs of maternal care and increase the probability of offspring survival through improved vigilance and defence (Roulin, 2002). The majority of rodent communal nests are thought to consist of related individuals due to the high degree of cooperation required when living in such a system and the fitness benefits gained when helping to rear related individuals (Gerlach & Bartmann, 2002; Hayes, 2000; Roulin, 2002). Although house mice sometimes rear their offspring in a solitary nest, lifetime reproductive success has been shown to improve when females communally rear their pups, particularly if they nest with a relative (Konig, 1994a; Konig, 1994b). However, there is increasing evidence that female competition can result in reproductive suppression amongst group members, or infanticidal behaviour after birth of a litter (Palanza & Parmigiani, 1994; Roulin, 2002; Weber & Olsson, 2008); female age can also determine reproductive skew among sisters (Rusu, 2004; Rusu & Krackow, 2004). The first litter born may be at risk if the second female is in the final days of gestation, as the risk of maternal aggression and infanticidal behaviour increases (Rusu & Krackow, 2004). Giving birth synchronously has been suggested as a strategy to protect against infanticide, as both

roaming males and female social partners may not be able to distinguish between offspring (Maestripieri & Alleva, 1991; Poikonen *et al.*, 2008). Reproductive success is also likely to decrease with increasing group size due to increased reproductive skew between group members (Konig, 2006; Konig & Lindholm, 2012), and reproductive suppression through hormonal influence (Hurst, 2005; Wasser & Barash, 1983). Despite the potential for competition, remaining in the group as a non-breeder is likely to be preferable to rearing young alone in a solitary nest where intruders pose a threat and the benefits of thermoregulation are lost (Konig, 1994a).

Genetic components of competitiveness can be passed to offspring (Hager & Johnstone, 2006), and pups may be exposed to high levels of maternal androgens during gestation, which could result in more competitive offspring (Russell & Lummaa, 2009). Weaning weight of female house mice has previously been shown to influence the time of sexual maturation and litter size, as well as future competitive ability (Konig, 1989). Due to the potential for reproductive conflict between communally nesting females, it is possible that maternal strategies are used by female house mice, particularly in competitive environments. Females may reduce quantity of offspring born in competitive communal conditions compared to solitary conditions, and increase the quality of their pups to enable them to effectively compete with littermates. Sex ratio of litters may also be adjusted to increase the number of helpers for future reproduction (i.e. by giving birth to more daughters), or to enhance competitive ability of their offspring (i.e. by giving birth to heavier and potentially more aggressive sons).

As reproductive success may be increased when related females communally rear offspring (Konig, 1994b), females may prefer to nest with social partners more genetically similar to themselves, resulting in reduced levels of competition. Major urinary proteins (MUPs) have been shown to play an important role in group formation of wild house mice (Hurst *et al.*, 2001a), as individuals express unique MUP profiles as a signature of identity (Beynon & Hurst, 2003). House mice have been shown to use MUP sharing to avoid inbreeding (Sherborne *et al.*, 2007) as unrelated individuals are likely to express different MUP patterns (Beynon & Hurst, 2004). The amount of competitive behaviour performed between paired individuals may also relate to MUP profile as male mice were found to react less aggressively towards the odour of a MUP-similar brother than a MUP-dissimilar brother (Hurst *et al.*, 2001b). In Chapter 4 I showed that MUP patterns remained relatively consistent in female house mice, but there has been no evidence to suggest that the degree

of MUP sharing between female pairs influenced the intensity of competitive behaviour observed (Chapter 3). Although unrelated individuals have been used throughout my experiments, there is still a degree of MUP peak sharing between unrelated individuals and females may use these cues when selecting a nesting partner. It is therefore interesting to consider whether MUP sharing influences total or differential reproductive success under communal conditions.

Due to the costs associated with offspring care and the high energetic demands of lactation, there is potential for competition over maternal care in house mice. If communally breeding females are related, the costs associated with non-offspring nursing are thought to be offset through indirect fitness benefits (inclusive fitness) as females are helping to rear younger siblings, nieces, nephews, and cousins etc. (Manning *et al.*, 1995). However unrelated females also perform non-offspring nursing (Manning *et al.*, 1992), which does not provide the same degree of fitness benefits as nursing related young. In addition, non-offspring nursing can have negative physiological effects on an individual level; for example if females do not equally care for young then demand may be increased for the female providing more milk (Konig *et al.*, 1988). Milk quality may also be affected if females increased the amount of nursing they perform, which would then negatively impact on own offspring (Knight *et al.*, 1986). Inter-litter intervals can also be affected when females care for large litters or perform lactation for longer periods than anticipated (Fuchs, 1982; Manning *et al.*, 1995).

Communally nesting females do not appear to distinguish between offspring when providing communal care, as they retrieve all pups present in the nest and nurse them indiscriminately (Manning *et al.*, 1995). The consequences of losing a litter through infanticidal behaviour may also affect the stability of the social group; it is not known if affected females would remain at the nest site and care for her partner's offspring or refrain from communal nursing (Konig, 1994a). If one or more females reduced the amount of communal care they provided, then it could have serious negative implications for their own offspring, unless the social partner compensated for this loss (Konig, 2006). Subordinate female wood mice (*Apodemus sylvaticus*) have previously been shown to perform more time nursing pups than dominant social partners (Gerlach & Bartmann, 2002), although the division of communal nursing in house mice has rarely been investigated (Konig, 2006; Konig & Lindholm, 2012).

6.2.3 Experimental aims

As house mice have previously been shown to compete (Palanza *et al.*, 1996; Rusu & Krackow, 2004) and are known to reproduce rapidly (Berry, 1981), they are the ideal species in which to investigate the effects of female competition on reproductive output in the communal nest. In this chapter I investigate if competitive ability of the mother predicts litter size at birth, growth rates and offspring survival in the communal nest. I also investigate if the effects of competitive ability (if present) are exaggerated in communal nests compared to solitary breeding conditions. MUP similarity is also examined to determine if the degree of MUP peak sharing between females influences reproductive success in the communal nest. As less competitive females were mounted first in the previous chapter, I investigate if birth order is skewed between female partners and if pup survival is negatively affected as a consequence. Time spent in proximity to pups is used as a measure of maternal care to establish if there is a difference between more and less competitive females. Finally, I consider if the rearing environment or maternal competitive ability affects subsequent offspring reproductive success.

6.3 Methods

6.3.1 Animals

Female mice used in this experiment were reproductively inexperienced and aged approximately 3 and 6 months old (n = 70). Subjects were expected to show some competitive behaviour at the time of introduction to an unfamiliar, unrelated female social partner (see Chapter 3). As age had previously been shown to be an important predictor of competitive behaviours and reproductive output in experimental studies (Rusu, 2004), 2 groups of unrelated and unfamiliar female pairs were established: age-matched pairs at 3 months of age (n = 15 pairs) and age-difference pairs aged 3 and 6 months (n = 16 pairs). Individuals were classed as unrelated if they did not share more than 1 great-grandparent. Prior to testing all animals were housed in conditions described in Chapter 2, Section 2.1. Females were fitted with a RFID tag and their tail or fur marked for visual identification using the methods described in Chapter 2, Section 2.2. Breeding males were also reproductively inexperienced, unrelated to breeding females and aged approximately 3 to 8 months old (n = 70).

6.3.2 Experimental procedure

6.3.2.1 Solitary breeding

To examine reproductive success of females in the absence of competition (and to ensure that all females were capable of reproduction), females were housed with sexually mature males and allowed to breed and rear litters prior to competitive female interaction. Although first litters tend to be smaller than subsequent litters in wild house mice (Konig & Markl, 1987), it was important to determine if the failure to reproduce in the communal nest was a result of infertility or as a result of nesting with a competitive social partner. Therefore if competition had no negative implications on reproductive output, I predicted that communal litter size would be at least equal to that observed in the first solitary litter. If females did not successfully breed or performed infanticidal behaviour after they gave birth then they were not used in the second part of the experiment.

Four days prior to solitary breeding, females were weighed and soiled bedding from an unfamiliar and unrelated male was added to home cages to stimulate oestrus (see Chapter 2, Section 2.4). Males were also provided with soiled bedding from an unfamiliar and

unrelated group of females in their home cages to stimulate reproductive behaviour when introduced to female pairs (Cheetham *et al.*, 2007). On the day of male-female introduction, females were transferred to a clean MB1 cage containing a plastic nest box, substrate, nest material, a cardboard tube and *ad. lib.* access to food and water (as described in Chapter 2, Section 2.1). Males were introduced to the cage and pairs monitored for approximately 4 hours for aggressive behaviour (chasing, attacks or fights). All pairs were checked daily and food and water topped up. Two weeks after introduction females were checked every 1 to 2 days for signs of pregnancy. Once females had gained more than 5 g, had visible nipples and a protruding stomach then the male was removed and returned to the stock room in a clean M3 cage. Females were transferred to a clean MB1 cage with the nest box they had previously used. A handful of soiled bedding from their previous cage was also added to the new cage to ensure they could still encounter the male's odour, as this has been shown to maintain pregnancy in house mice (Kumar & Dominic, 1993).

On the day of birth (post natal day 1), female weight and latency to birth was recorded as well as litter size and litter weight. When weighing litters, special precautions were taken to ensure no foreign scents were transferred to pups. Using a clean pair of gloves for each litter, soiled substrate and nest material from the home cage were rubbed over the surface of each glove. Pups were then carefully removed from the cage and placed on a handful of previously weighed home cage bedding on the scale, before quickly being returned to the home cage. Females were observed for approximately 10 minutes following this procedure to ensure that they continued to care for the offspring and did not perform infanticide due to a change in scent or through disturbance (Hurst, 2005). On post natal day 15, females were observed for 4 hours to measure the total time they spent in contact with their pups (see Section 6.3.2.4). At weaning (post natal day 24) pups were removed from the female cage, counted and weighed. Pups were then housed according to gender, with males housed individually in clean M3 cages and females with their sisters in groups of 2 to 4 individuals in MB1 cages.

As 8 females did not successfully breed they were excluded from the experiment, resulting in 62 females available for use. Subject females were transferred to clean MB1 cages for approximately 4 days once their litters had been weaned. The remaining 8 females were re-housed in groups of 2 to 3 and returned to the stock room.

6.3.2.2 Competitive female interaction

This part of the experiment was conducted in 2 blocks, each lasting 4 months. The first block consisted of 8 pairs of age-matched females (3 months) and 8 pairs of age-difference females (3 and 6 months), and the second block consisting of 7 pairs of age-matched and 8 pairs of age-difference females. To ensure all females were tested during the red light phase, 8 pairs were introduced in a single test day with tests conducted from 09:00 to 18:00 hours. Therefore in block 1, 4 pairs of age-matched females and 4 pairs of age asymmetry females were introduced on 1 day, and the remainder introduced on the subsequent day.

Four days prior to competitive interaction, females were weighed and urine sampled using the recovery method (Chapter 2, Section 2.3). Soiled bedding from an unfamiliar and unrelated male was added to their home cages to stimulate oestrus (see Chapter 2, Section 2.4). On the test day females were introduced to an unfamiliar and unrelated female social partner in a test arena as described in Chapter 2, Section 2.6. At the end of the 30 minute test, females were individually transferred to a clean MB1 cage for approximately 2 to 3 hours. These cages contained all of the standard housing enrichment as described in Chapter 2, Section 2.1.

If females were excessively aggressive during the 30 minute trial (see ethical rule in Chapter 2, Section 2.7), the test was stopped and females were housed in a specially designed divided cage overnight (as described in Chapter 5, Section 5.3.2.1). If this occurred then females were re-introduced using the same methods as described above on the next day. DVD recordings of behaviour during the tests were watched blind to the identity of the mice and the frequency of competitive behaviours (attack, chase and fight) and submissive behaviours (retreat, submissive posture) were recorded (see Chapter 2, Table 2.1).

Approximately 4 hours after being introduced to their social partner, females were transferred to a semi-naturalistic test arena (described in Chapter 2, Section 2.8) in their MB1 cages with the lid closed for 30 minutes to habituate to the test room. After the habituation period, cage lids were removed and placed next to the cage, enabling females to freely interact with one another and access food and water *ad. lib.* from the food hopper on the cage lid. Night vision cameras were suspended from brackets above the melamine enclosures to capture behaviour which was recorded onto DVD in an adjacent room (see Chapter 2, Section 2.8). Observations could be made during the test to ensure that no

excessive aggression occurred during the trials (see ethical note in Chapter 2, Section 2.7). Filming commenced at the time of lid removal for 4 hours. Female latency to approach her social partner was recorded as well as the frequency of competitive and submissive behaviours for the duration of the experiment (see Chapter 2, Table 2.1). At the end of the experiment females were left in the test arena and cage lids placed onto the cage base at a right angle to enable females to freely enter and leave the cages. Two metal sheets (12 x 6 x 6 cm) were placed on top of the cage lid to provide areas of cover during the white phase of the light cycle.

All female pairs were checked 3 times a day and observed for approximately 15 minutes to ensure no excessive aggression occurred between pairs. Four days after the competitive female interaction tests all females were removed from the melamine enclosure and checked for signs of injury to the face, body or fur using a clear Perspex handling tube as previously described. Females were then urine sampled using the recovery method, before being returned to their enclosure. Soiled bedding from an unrelated and unfamiliar male was then added to both open cages within the enclosure and to the centre of the enclosure to stimulate oestrus in both females.

6.3.2.3 Communal breeding

Breeding males used in this part of the experiment were used in Section 6.3.2.1., and so were reproductively experienced. However males were not matched with females that they had previously encountered or with their relatives. Males were therefore unrelated and unfamiliar to either female within the pair to which they were introduced. Four days prior to communal breeding, male subjects were provided with soiled bedding in their home cages from an unfamiliar and unrelated group of females to stimulate reproductive behaviour when introduced to the female pairs (as described in Chapter 2, Section 2.4). One week after competitive female interaction, males were weighed and placed individually inside the test enclosure within their M3 home cages with the lid closed for 30 minutes. Female competitive and submissive behaviours were recorded remotely to DVD (as previously described), as well as the latency to approach the male's cage. At the end of 30 minutes each male's cage lid was removed to enable all individuals to freely interact in the test arena. Recording continued for a further 3.5 hours and behaviour was observed to ensure that no excessive aggression occurred during the test period. If groups had to be interrupted more than 3 times over a 30 minute period then 1) the male was removed from

the arena and returned to his home cage overnight, and 2) females were individually enclosed within the 2 MB1 cages in the arena for 1 hour before removing the lids and observing interactions for 30 minutes for excessive aggression. Males were then re-introduced using the same methods as above on the next day. At the end of the test period all subjects were left in the test arena to freely interact for a minimum of 14 days.

All groups were checked daily to ensure they were freely interacting and that food and water were accessible from the 3 food hoppers on the cage lids. Two weeks after male introduction, females were captured in home cages or in a handling tube and removed from the test arena to check for signs of pregnancy. If at least 1 female in a pair had gained more than 5 g, had visible nipples and a protruding stomach then the male was removed from the enclosure and returned to the stock room in a clean M3 cage. Both females were then transferred to clear MB1 cages with substrate, 2 plastic nest boxes and nest material (as described in Chapter 2, Section 2.1) in preparation for birth. If neither female displayed visual signs of pregnancy then they were returned to their enclosure and checked again 2 to 3 days later.

Latency to birth, female weight, litter size and litter weight were all recorded on the day of birth (as previously described in Section 6.3.2.1). If both females had given birth on the same day then the combined litter was weighed and counted. On post natal day 15, both females were observed for 4 hours to measure the total time they spent in contact with the litter(s) (see Section 6.3.2.4). Cages were checked daily and the number of pups counted until the time of weaning at post natal day 24.

At weaning, all pups were removed from the nest, counted and weighed. Pups were housed according to gender, with males housed individually in M3 cages and females housed with their sisters in groups of 2 to 4 in MB1 cages (see Chapter 2, Section 2.1). If females had given birth on the same day then maternal identity could not be established. These pups were therefore humanely culled (as described in Chapter 2, Section 2.9) on post natal day 24 (i.e. weaning) and immediately frozen at -22^{o}C for parentage analysis at a later date (see Section 6.3.2.6 below). Female subjects were weighed and urine sampled using the recovery method, before being transferred to a clean MB1 cage and returned to the stock room.

6.3.2.4 Maternal behaviour

During rearing in solitary and communal litters maternal behaviour was observed for 4 hours on post natal day 15. Maternal behaviour was classed as time spent in close proximity to (within half an adult body length), or in contact with pup(s) in the communal nest when duration lasted longer than 5 seconds. To enable clear vision of the litter(s), excess nest material was removed from the MB1 home cage and the metal cage lid replaced with a clear Perspex lid perforated with holes (44.5 x 27.5 x 12.5 cm) approximately 1 hour prior to filming to allow females time to acclimatise to the change in the environment. To ensure females had *ad. lib.* access to food and water, approximately 12 food pellets were scattered in the base of the cage on top of the substrate material and water bottles positioned on specially adapted holders made of Perspex and wire. Cages were placed in the centre of melamine test arenas with night-vision cameras suspended from brackets above the arenas to capture behaviour remotely and record onto DVD in an adjacent room for 4 hours. At the start of the test, all pups were carefully removed from the nest box and placed at the opposite end of the cages. Latency to retrieve pups was then recorded as well as the time spent in proximity to pups. Time in proximity to pups has previously been used as a measure of maternal care in African striped mice (*Rhabdomys pumilio*) (Kinahan & Pillay, 2008) and is a measure that is relatively non-invasive and requires little disturbance to the cage. This method therefore should not affect maternal care behaviour performance or influence the risk of infanticide in this experiment. At the end of the test all nest material was replaced in the home cage and the Perspex lid replaced with the metal cage lid. Food and water was also returned to the food hopper in the cage lid. DVDs were watched blind to the identity of mice.

6.3.2.5 Reproductive output (offspring)

To assess the reproductive success of offspring born in this experiment, reproductive data were extracted from the mouse database at the Mammalian Behaviour and Evolution group at the University of Liverpool. At the time of weaning, offspring are given a unique identification number and entered onto the database along with the ID references of their parents. This enables users to trace relatedness between individuals when designing experiments and to identify the total number and sex ratio of offspring weaned by an individual. In addition to the database I also used data collected by another PhD student (Andrew Holmes), who bred some of the offspring produced in this experiment once they

had reached 4 months of age. Handling and breeding techniques were identical to those described in Chapter 2, Section 2.1 and Section 6.3.2.1 respectively. Litter mass and size were recorded on post natal days 1 and 24, as well as sex ratio of the litter. This information was then added to the data I collected from the central database for further analysis.

6.3.2.6 Genotyping procedure

Where females gave birth on the same day in the communal nest, maternal identity could not be established. At the end of the experiment, these offspring were culled and immediately stored at -22^{o}C, while female subject individuals were transferred to the stock room to enable them to be used for other experiments. At the time of each subject female's death, a 1 to 5 mm tail snip was taken from each individual for DNA extraction. Tail snips were also taken from each pup that had been stored in the freezer.

DNA extraction was carried out using a QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, West Sussex, UK), following the manufacturer's instructions precisely. To establish the haplotypes 3 microsatellite markers from across the MHC region on chromosome 17 were used, 6 microsatellite markers from across the MUP region on chromosome 4 were used, and 2 microsatellite markers from across the ESP region on chromosome 17 were used. These were selected from the Mouse Genome Informatics site (MGI 5.1.3). The forward primer for each of the 11 microsatellite markers was 5'-end fluorescently labelled with 6-FAM, PET, NED or VIC phosphoramidite. This allowed for multiple markers to be pooled into a single run.

Polymerase Chain Reaction (PCR) amplification was carried out by Amanda Davidson at the Protein Function Group, University of Liverpool and conducted in 10 µl reactions of 20 ng DNA, 0.5 µM primer and 5.0 µl of BioMix Red reaction mix (Biolin, London, UK). The PCR protocol steps were: an initial denaturation for 2 minutes at 95°C; 30 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C to 58°C (depending on the primer) for 2 minutes, and extension at 72°C for 30 seconds; and after the 30 cycles were complete a final extension at 72°C for 10 minutes. The PCR reactions were then diluted to 25- to 50-fold (depending on primer set) and multiplexed in formamide with GeneScan LIZ500 size standard (Applied Biosystems). Haplotype size was determined with an ABI PRISM 3100 DNA analyzer and GeneMapper v3.0 software (Applied Biosystems).

The resulting data was compiled in Microsoft Excel (2007). The output was grouped into MHC, MUP and ESP markers. Individuals had 2 alleles for each microsatellite marker. Parental triads were grouped together and 1 microsatellite marker was identified for each set of potential parents and used to establish pup parentage. The MUP microsatellite marker (VICNDS6) was used for 2 groups of animals while the MUP microsatellite marker (PET139) was used for 1 group. Offspring were then genotyped using the identified microsatellite marker as described above. Offspring parentage was then assigned based on possible parental alleles and own allele set.

6.3.3 Data analysis

Where data did not meet parametric assumptions, a log transformation was applied. Non-parametric statistics were used for analysis when data could not be normalised by transformation. Where parentage of offspring could not be determined, pairs were excluded from analysis of reproductive output. All statistical tests were carried out using SPSS software v20 and graphs produced in Microsoft Excel (2007).

A multivariate linear regression was used to determine if female age, body mass, urinary testosterone or protein significantly related to reproductive output in the solitary nest. Paired t-tests were used to examine the differences in reproductive output on post natal days 1 and 24 for more and less competitive females, and to examine differences in litter mass and sex ratios. The relative change in reproductive output between the 2 nest environments were then compared between more and less competitive females using a Wilcoxon paired test.

Paired t-tests were used to examine the differences in absolute reproductive output between more and less competitive females in the communal nest. Chi-squared tests were used to compare the frequencies of more and less competitive females that gave birth in the communal nest. To examine relative differences in reproductive output on post natal days 1 and 24 between more and less competitive females, I conducted univariate general linear models (GLMs), adding age group as a fixed factor, and body mass and competitive score asymmetries as covariates. Paired t-tests were used to look for differences in sex ratio and weight gain of litters weaned by more and less competitive females (and older and younger females in the age-difference group). Pearson's correlation tests were used to examine the

relationship between MUP peaks shared and the number of pups weaned in the communal nest for more and less competitive females.

Paired t-tests were used to compare latencies to birth between more and less competitive females in the communal nest, and to compare the reproductive output and weight gain of pups born to females giving birth first and second. Maternal behaviour data were also analysed using paired t-tests, to identify if more or less competitive females spent more time in contact with the communal litters, and if there was a difference in the time females spent with a solitary or communal litter. In these tests, the number of pups was controlled for by dividing the total amount of time females spent in contact with 1 or more pup by the number of pups present in the nest.

Finally a repeated measures GLM was used to compare the average litter size (over 3 successive litters) of daughters born to more and less competitive females in both solitary and communal nests during this experiment; competitive rank of dams and nest environment were therefore added as fixed factors in this model.

6.4 Results

6.4.1 Influence of female characteristics on reproductive output in a non-competitive environment

Prior to competitive interaction, females gave birth to a litter in solitary nests and therefore reproductive output was not affected by the presence of a female social partner. I therefore investigated if there were physiological characteristics that could explain variation in reproductive output, in the absence of competition. The results of a multivariate linear regression revealed that female body mass positively affected the number of pups present on both post natal days 1 and 24 (Table 6.1). No other significant relationships were found between reproductive output and female age, anogenital distance or urinary testosterone (Table 6.1).

6.4.2 Influence of nest environment on absolute reproductive success

Four days after competitive interaction, female pairs were introduced to a sexually mature male to breed communally with their competitive social partner (males were removed shortly before the first birth). In the communal nest, reproductive output on post natal day 1 was reduced for both competitive and less competitive females compared to previous breeding success in solitary conditions, despite the prediction that secondary litters tend to be larger than the first (more competitive $t_{[28]} = 3.896$, $p = 0.001$; less competitive $t_{[28]} = 5.255$, $p < 0.001$; Figure 6.1). The number of pups weaned in the communal nest was also reduced for more competitive females ($t_{[28]} = 6.057$, $p < 0.001$) and less competitive females ($t_{[28]} = 6.730$, $p < 0.001$) compared to the solitary nest. The relative decrease in reproductive output in the communal nest was not significantly different for more or less competitive females at post natal day 1 ($t_{[28]} = 0.988$, $p = 0.331$) or at post natal day 24 ($t_{[28]} = 0.000$, $p = 1.000$).

Total litter mass at weaning was reduced for more competitive females in the communal nest ($t_{[15]} = 2.724$, $p = 0.016$), but not for less competitive females ($t_{[12]} = 1.079$, $p = 0.302$) suggesting that despite a decrease in litter size, litter mass was maintained in the communal nest for less competitive females. The relative change in litter mass from solitary to communal conditions was not significantly different for more or less competitive females ($Z = -0.210$, $n = 8$, $p = 0.833$). There was also no difference in the sex ratio of litters

weaned between the solitary and communal conditions (more competitive $t_{[15]} = -0.348$, $p = 0.732$; less competitive $t_{[12]} = 0.590$, $p = 0.566$).

Overall, a higher frequency of more competitive females gave birth in the communal nest compared to less competitive females (81% compared to 55%; $\chi^2 = 4.724$, $df = 1$, $p < 0.050$). However, of those females that gave birth there was no significant difference in the frequency of competitive or less competitive females that successfully weaned offspring (72% to 88%; $\chi^2 = 1.584$, $df = 1$, $p > 0.050$). Reproductive failure for both females within a pair only occurred on 3 instances.

Table 6.1 – Summary results of a multivariate linear regression investigating the effects of female age and physiological characteristics on reproductive output in solitary breeding conditions (post natal days 1 and 24).

Dependent	Independent	β	t	F	p
Pups present (PND 1)				**$F_{[4,54]}$ = 4.063**	**0.006 (R^2 = 0.245)**
	Age (days)	-0.004 (±0.005)	-0.744		0.461
	Anogenital distance	0.082 (±0.960)	0.085		0.932
	Body mass	0.373 (±0.101)	3.685		0.001***
	Testosterone	0.001 (±0.007)	0.221		0.826
Pups present (PND 24)				**$F_{[4,54]}$ = 4.340**	**0.004 (R^2 = 0.258)**
	Age (days)	-0.004 (±0.005)	-0.805		0.424
	Anogenital distance	0.164 (±0.949)	0.173		0.863
	Body mass	0.382 (±0.100)	3.824		<0.001***
	Testosterone	0.002 (±0.007)	0.359		0.721

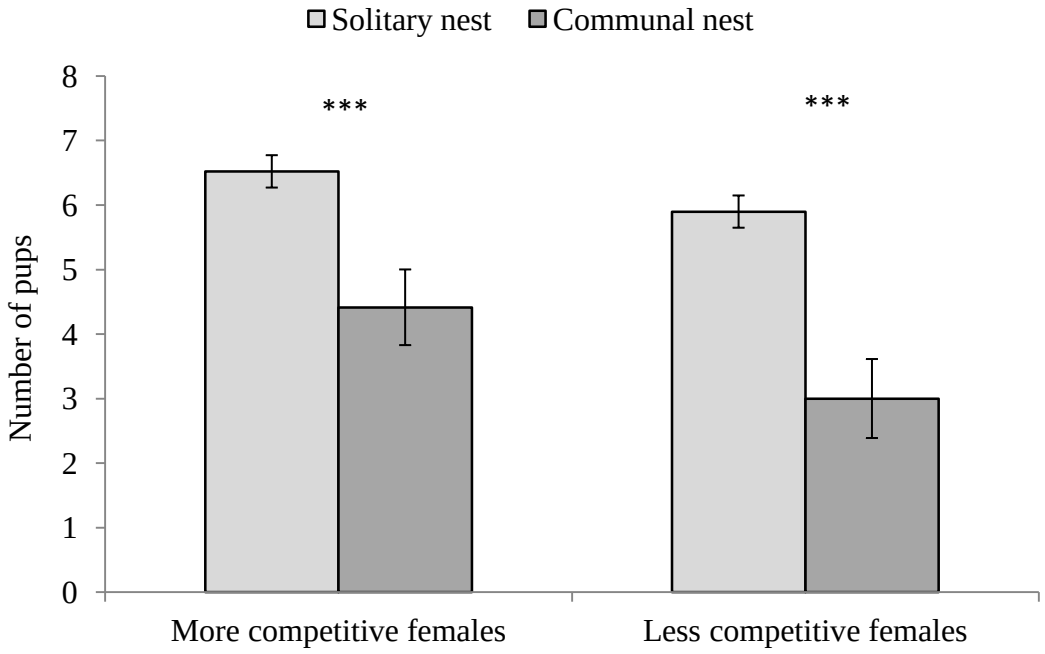


Figure 6.1 – Mean (±se) number of pups present on post natal day 1 for competitive and less competitive females in solitary and communal breeding conditions (* p < 0.001).**

6.4.3 Influence of age asymmetry on relative reproductive success between communally nesting social partners

As younger females had previously been shown to display more submissive behaviours when introduced to an unrelated and unfamiliar conspecific during interaction tests (Chapter 3), I investigated if older females had reproductive advantages in the communal nest. I also investigated if reproductive skew differed between treatment groups (i.e. when females were paired with a same aged partner at 3 months, or a different aged partner at 3 or 6 months).

Reproductive skew (i.e. difference in reproductive output) was significantly different between age groups on post natal day 1 ($F_{[1,25]} = 5.231$, $p = 0.031$) and while there was no significant relationship between reproductive skew and competitive score asymmetry ($F_{[1,25]} = 0.568$, $p = 0.458$), there was a significant positive relationship between reproductive skew and body mass asymmetry ($F_{[1,25]} = 4.584$, $p = 0.042$). When females were paired with a different aged partner (aged 3 or 6 months), heavier females were more likely to have increased reproductive output in the communal nest ($F_{[1,12]} = 5.270$, $p = 0.041$), however there was no significant relationship between competitive score asymmetry and reproductive skew ($F_{[1,12]} = 0.816$, $p = 0.384$). There was a non-significant trend for older females to be heavier than younger females ($t_{[30]} = 1.920$, $p = 0.064$), suggesting the age and weight were potentially confounded in this group. When females were paired with a similar aged partner at 3 months, there was no significant difference in reproductive skew between partners ($F_{[2,11]} = 0.096$, $p = 0.909$). There were also no significant relationships between reproductive skew and competitive score asymmetry ($F_{[1,11]} = 0.054$, $p = 0.820$) or differences in body mass ($F_{[1,11]} = 0.130$, $p = 0.725$).

There was no difference between treatment groups in reproductive skew at weaning on post natal day 24 ($F_{[1,25]} = 0.256$, $p = 0.617$). Body mass asymmetry between females positively related to the amount of reproductive skew ($F_{[1,25]} = 4.487$, $p = 0.044$), suggesting that heavier females weaned more offspring, but there was no relationship between reproductive skew and competitive score asymmetry ($F_{[1,25]} = 0.030$, $p = 0.863$).

Survival rates of pups between females was not significantly different between age-matched and age asymmetry pairs ($F_{[1,8]} = 0.067$, $p = 0.803$), neither was there a relationship with body mass asymmetry between females and survival differences ($F_{[1,8]} =$

0.807, p = 0.395) or competitive score asymmetry ($F_{[1,8]}$ = 3.316, p = 0.106). However, when survival rate of offspring was combined for both females in a pair, survival was significantly higher for female pairs in the age-difference group (age-difference 81.8 $\pm$ 1.0%; age-matched 57.5 $\pm$ 1.1%; U = 57.500, n = 28, p = 0.045).

No significant differences were detected in total litter mass on post natal days 1 or 24 for older or younger females within the age-difference group (PND 1 $t_{[6]}$ = -0.847, p = 0.430; PND 24 $t_{[6]}$ = 0.602, p = 0.569). There was however a non-significant trend for older females pups to gain more weight from post natal days 1 to 24 compared to younger females pups (average pup weight gain for older dams 11.28 $\pm$ 0.51 g; younger dams 9.55 $\pm$ 0.71 g; $t_{[6]}$ = 2.188, p = 0.071). The rate in which pups gained weight did not appear to be affected by the nest environment as there was no difference between solitary and communal pups for older females ($t_{[9]}$ = -0.670, p = 0.520) or younger females ($t_{[7]}$ = -0.990, p = 0.355).

6.4.4 Influence of birth order on reproductive success in the communal nest

As less competitive females were the first to mate in a semi-naturalistic environment in a previous experiment (Chapter 5), it was possible that latency to birth could have been shorter for less competitive females in the communal nest. However, when both females produced a litter in the communal nest, the difference in latency to birth between female partners was not significantly reduced for less competitive females ($t_{[11]}$ = -0.151, p = 0.882).

Reproductive output at post natal days 1 and 24 appeared to vary according to birth order in the nest. Three age-matched pairs and 3 age-difference pairs gave birth on the same day, and all but 1 age-matched pair weaned all pups present on post natal day 1 (both females lost all pups). Three age-matched pairs and 5 age-difference pairs gave birth asynchronously (> 12 hours apart), and of those only 4 age-difference pairs weaned all pups present on post natal day 1. Only 1 female successfully gave birth to a litter in 3 age-matched pairs and 3 age-difference pairs, with only 1 age-difference female successfully weaning all pups.

On occasions when communal litters were born at least 12 hours apart, the first female to give birth had fewer pups present on post natal day 1 than the second female ($t_{[7]}$ = -2.479, p = 0.042; Figure 6.2). There was also a non-significant trend for the first female to give

birth to wean fewer pups than the second female ($t_{[7]}$ = -2.110, p = 0.073; Figure 6.2), although pup survival was not significantly different between the first and second female to give birth (Z = -1.095, n = 8, p = 0.273). Average pup weight was not significantly higher in first born litters ($t_{[6]}$ = 0.083, p = 0.937) and total litter mass was not significantly different for first or second born litters ($t_{[6]}$ = -1.536, p = 0.175). Females that gave birth first in the communal nest did not wean more male offspring compared to the second female ($t_{[6]}$ = -1.045, p = 0.336), but there was a non-significant trend for the first litter born to gain more weight from post natal days 1 and 24 (on average per pup) than the second litter born ($t_{[6]}$ = 2.113, p = 0.079).

6.4.5 Influence of MUP peak sharing between competitive females on reproductive output in the communal nest

There was a significant positive correlation between MUP peak sharing of female social partners and the number of pups present on post natal day 1 and 24 by less competitive females (PND 1 r = 0.593, n = 29, p = 0.001; PND 24 r = 0.706, n = 29, p < 0.001), suggesting that reproductive output was increased when less competitive females shared relatively more MUP peaks with their social partner. There was also a positive but non-significant correlation between MUP peak sharing and the number of pups weaned by more competitive females in the communal nest (r = 0.347, n = 29 p = 0.065), however there was no correlation between MUP sharing and pups present on post natal day 1 (r = 0.087, n = 29, p = 0.655).

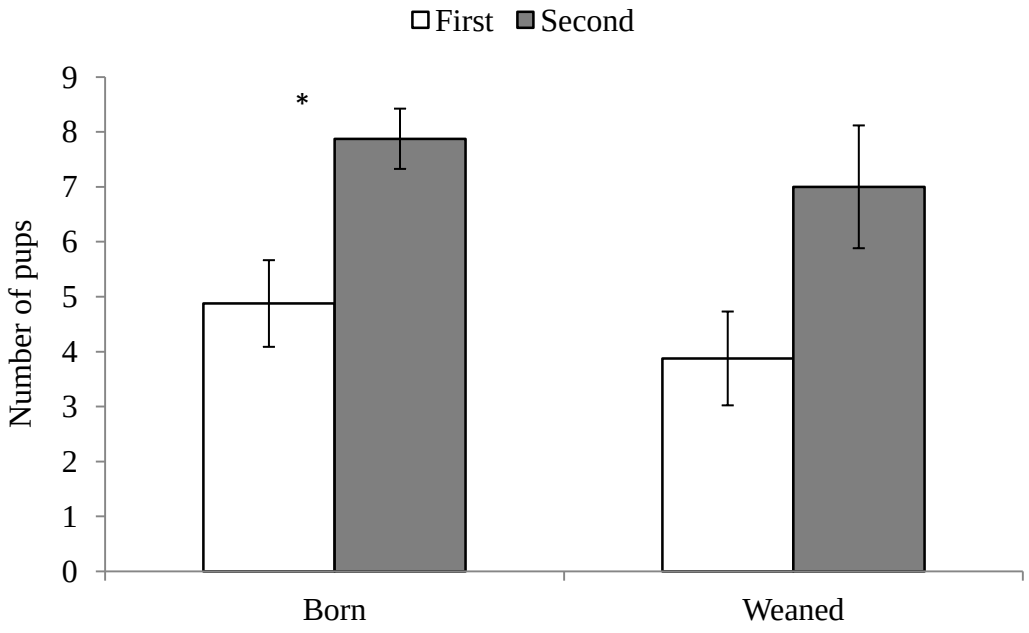


Figure 6.2 – Differences in mean (±se) pups born and weaned to females giving birth first and second in the communal nest ($p < 0.050$)

6.4.6 Maternal care division by competitive females in the communal nest

As there was evidence of infanticidal behaviour and a reduction in reproductive output in the communal nest, I investigated if the division of maternal care was skewed between competitive female partners. A paired t-test revealed that there was no difference in the total amount of time that more competitive and less competitive females spent with pups in the communal nest when both females had offspring present ($t_{[8]}$ = -0.725, p = 0.489), however the average amount of time females spent in proximity to the litter (measured as time per pup in the nest) was reduced for more competitive females in the communal nest compared to solitary breeding conditions ($t_{[7]}$ = 2.643, p = 0.033; Figure 6.3). There was no difference in time spent in proximity to pups by less competitive females in the solitary or communal nests ($t_{[7]}$ = 1.759, p = 0.122; Figure 6.3). When only one female gave birth in the communal nest there was no difference in the total amount of time either female spent in proximity to pups ($t_{[6]}$ = -0.687, p = 0.518) or in the average amount of time per pup present in the nest ($t_{[6]}$ = -0.835, p = 0.436). The non-lactating female did not significantly reduce the total amount of time in proximity to her own pups in the solitary nest compared to her partners pups in the communal nest ($t_{[3]}$ = 0.414, p = 0.707), or the average time per pup present ($t_{[3]}$ = -0.276, p = 0.800).

6.4.7 Influence of competitive rearing environments and competitive rank of dams on offspring reproductive success

As more competitive females were likely to obtain advantages in pup growth in the communal nest, I investigated if there were potential fitness benefits for offspring born to more competitive mothers and/or if rearing environment influenced reproductive success at maturity.

A univariate GLM was used to test if females born in a competitive (i.e. communal litter) environment had higher average reproductive output (across 3 litters) compared to those born in a less competitive (i.e. solitary litter) environment. Competitive ranks of dams was also included in the analysis, to test if females born to more competitive mothers had higher reproductive output than females born to less competitive mothers. The results revealed that average litter size was higher for females born to a more competitive mother ($F_{[1,10]}$ = 10.581, p = 0.009), however there was no effect of nest environment on average litter size ($F_{[1,10]}$ = 0.681, p = 0.433). In addition, the average sex ratio of litters was

examined to determine if females reduced the number of female offspring they produced in competitive environments. The results revealed that litters were significantly more male biased if females had been born in a competitive communal nest environment compared to solitary environment ($F_{[1,9]} = 6.691$, $p = 0.029$), however the competitive rank of the dam did not appear to significantly influence average sex ratio ($F_{[1,9]} = 0.004$, $p = 0.951$).

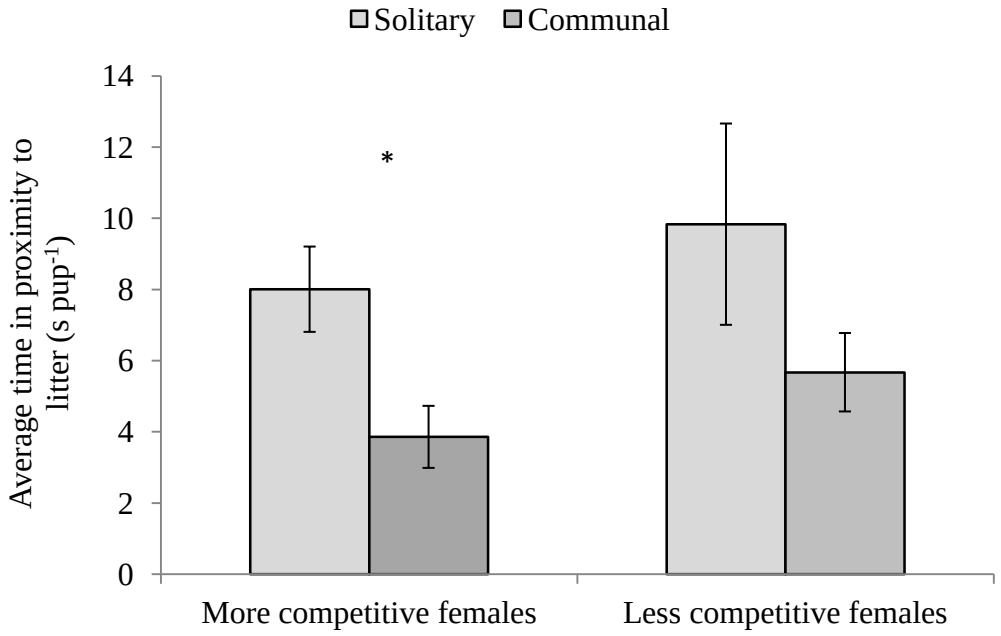


Figure 6.3 – Mean ± se time females spent with communal litters (seconds per pup) in the solitary and communal nest ($p < 0.050$).

6.5 Discussion

Overall reproductive output was reduced in the communal nest compared to solitary breeding, despite the expectation that secondary litters tend to be larger than the first born. This suggests that breeding with a social partner in a competitive environment negatively affected reproductive success for both more and less competitive females. As expected, larger females were also more likely to bear larger litters that survived until weaning age in the solitary nest. In the communal nest, heavier females were also more likely to have higher reproductive output, although this result may have been confounded by age. MUP peak sharing between females also positively influenced reproductive output at post natal day 24 for both females in the communal nest. Reproductive output did not appear to be affected by testosterone or anogenital distance when females reproduced prior to competitive interaction with a social partner. Therefore there is no suggestion that traits typically associated with aggression (Frank, 1986; Packer *et al.*, 1995) are sufficiently detrimental to wild house mice breeding in non-competitive environments. There was also evidence to suggest that daughters born to more competitive females had increased reproductive success in later life. Litters were also male biased if females had been reared in communal nests.

6.5.1 Reproductive consequences of competition in the communal nest

Reproductive output was reduced in the communal nest for both more and less competitive females on post natal day one, despite the expectation that second litters should be slightly larger for all females. It would have been interesting to compare reproductive success in two blocks, with half of the test females nesting communally and half nesting in solitary conditions in the first block before they alternated conditions for the second block. However it would have been difficult to distinguish if reproductive failure during communal nesting was related to competition between female pairs or for other physiological reasons. By manipulating the rearing condition order for all females I was also able to control for previous reproductive experience, which has been shown to influence competitive behaviour between unfamiliar and unrelated females at introduction (Chapter 3).

Females paired with a different aged partner were more likely to have pronounced reproductive skew, as heavier (and possibly older) females were more likely to produce

larger litters. Pup survival was lowest for female pairs that were housed with an aged-matched partner at three months of age, although pup loss also occurred in age-difference pairs. There was strong evidence that infanticidal behaviour was performed either by the mother or social partner. Whole and partial litters were consumed, regardless of birth order or the pregnancy status of the social partner, suggesting that maternal aggression alone could not fully explain the occurrence of pup death (Maestripieri, 1992; Maestripieri & Alleva, 1991; Palanza & Parmigiani, 1994). In previous experimental studies with house mice, infanticidal behaviour was more likely to be performed by pregnant females when their partners gave birth approximately one to four days before them; consequently two lactating females were available in the nest site to care for a single litter, and the infanticidal female was provided with an additional source of nutrition (Rusu & Krackow, 2004). Dams may also perform infanticide if they perceive a threat to their young which would enable them to immediately reproduce again, but the time and energetic costs already invested in gestation could outweigh the benefits gained from such behaviour (Hager & Johnstone, 2004). Although cages were checked on a daily basis in this experiment, there was no way to confirm that the number of pups present on post natal day one were the total number of pups a female had given birth to. Litters may have been reduced by mothers in response to the perceived competitive environment, however as the behaviour was never observed during this experiment it is difficult to establish the identity of the infanticidal individual.

Female house mice generally give birth on the same day (Konig, 1993; Konig, 1994a; Konig & Lindholm, 2012; Manning *et al.*, 1995) and as a result, benefit from increased survival of young as the risk of infanticidal behaviour is reduced (Hayes, 2000; Palanza *et al.*, 1996). Some pairs in this experiment gave birth on the same day and reproductive success was relatively high for the majority. However there were many instances were birth was either asynchronous or only one female successfully reproduced. When females gave birth asynchronously, the second litter contained more pups on post natal day one, so it is possible that litter size of the first litter was reduced as a result of infanticide (see above). Alternatively, the reduction in litter size could have been a maternal strategy by the first female to give birth, although this is unlikely as her pups were not significantly larger at birth compared to the second litter born. There was a trend for first born litters to gain more weight than the second litter, suggesting that females provide their pups with a competitive advantage by giving birth first. Therefore individual pups are more likely to

monopolise access to milk from lactating females, possibly simultaneously as the number of available teats exceeds pup number (Mock & Parker, 1997; Parker *et al.*, 1989).

Although I have previously shown that less competitive females were mated before their social partners (Chapter 5), less competitive females in this experiment did not give birth first. Latency to birth was calculated from the time that females were paired with a male, to the day that pups were present in the nest. House mice have been reported to have average gestation lengths of 19 to 21 days when not simultaneously lactating (Berry, 1970). In this experiment both solitary and communal litters were born 24 days (on average) after pairing with a male for both more and less competitive females. If less competitive females were mated during the first oestrus cycle and more competitive females mated on the second cycle, it is possible that more competitive females reduced their gestation length during this time period. Further tests would be needed to clarify this hypothesis, using the length of time between the presence of copulatory plugs and birth as a measure of gestation length. As fewer less competitive females gave birth in the communal nest in this experiment, it is also possible that mounting attempts did not result in successful copulation, or that pregnancy was not maintained as a result of social stress (Creel, 2001; Wasser & Barash, 1983). All females were monitored for weight changes between the time of male introduction and 14 days later (then daily from 15 days). Almost all females gained weight after this time, but for six less competitive females there was no evidence that the females had given birth. Subsequently a higher frequency of more competitive females gave birth, which suggests that resorption may have occurred. During resorption the pregnant female gains nutrients from the absorbed foetuses and immediately reduces her energetic demands, leaving her able to reproduce again (Mock & Parker, 1997), however it can occur as a result of stress and/or harassment from the more competitive female (Clutton-Brock *et al.*, 2008; Creel & Waser, 1994), which puts the less competitive female at a disadvantage unless she can successfully reproduce again. As females were not given the opportunity to leave the test arena during the experimental period, it is impossible to say whether unsuccessful females would have remained at the nest sites with their social partners after this time. Each female pair in this experiment had access to two nest boxes in the test arena and were observed nesting together on the majority of observational checks performed (>90%). Therefore it appears that either females are motivated to remain in contact with their social partner when contained in a relatively large arena, or that they are

unable to restrict their social partner from accessing their space if they did choose to nest alone.

6.5.2 Maternal behaviour between competitive partners

Maternal behaviour in this experiment was measured as the average time females spent in proximity to the communal litter (for each pup present). Despite the reduction in communal litter size and the presence of another lactating female, less competitive females did not reduce the average amount of time they spent in contact with pups, unlike subordinate wood mice (Gerlach & Bartmann, 2002). Less competitive females may adopt a strategy to enhance offspring quality after birth by spending more time caring for them. Indeed, the total litter mass of their communally reared pups at weaning was no different from the total weaning mass in the solitary nest, despite the reduction in litter size. It is not known if females can discriminate between their own offspring and their partner's when performing maternal care duties, or even if they are able to selectively nurse their own young when all littermates are scrambling for access to milk (Roulin, 2002), but the offspring of less competitive females may gain advantages from their mother's motivation to perform maternal care. There was however some evidence to suggest that more competitive females reduce the amount of time they spend in proximity when litter size was controlled for. This would potentially increase demand for milk for both litters and could affect milk quality in less competitive females (Knight *et al.*, 1986). Interestingly, when only one female gave birth in the communal nest there was no difference in the amount of time either partner spent in proximity to pups, suggesting that both females were motivated to perform parental behaviours, even if they were not lactating. Motivation to respond to pup calls is thought to be independent of the stage of oestrus (Ehret & Schmid, 2009) and is performed by both experienced and inexperienced female rats in the communal nest (Seip & Morrell, 2008). As maternal behaviour was measured on post natal day 15, both females had continuous exposure to the litter. Reproductively inexperienced female rats have previously been shown to perform maternal behaviour when constantly exposed to pups for two to nine days (Seip & Morrell, 2008), therefore the non-lactating partner may be motivated to respond to pup calls. As females were confined to the same cage as their social partner, there was no opportunity for dispersal and therefore non-lactating females could not leave to find an alternative nest site. In a confined environment it may pay-to-stay and help to rear the offspring of the social partner as this may improve

the social relationship between the females and improve future reproductive opportunities (Gilchrist, 2007).

6.5.3 Influence of MUP peak sharing on reproductive output

MUP peak profile sharing between female pairs was used as a measure of perceived similarity and may be used along with MHC by females to assess relatedness (Holmes, 2012). In this experiment less competitive females were found to have higher reproductive output in the communal nest when MUP profiles of their social partners were more similar. More competitive females also benefit from nesting with a more similar partner, but only for reproductive output on post natal day 24. Although MUP profile similarity was not found to influence competitive behaviour at introduction (Chapter 3), it is possible that female pairs with similar MUP profiles compete less intensely during gestation. Consequently, the stress response may not have been as pronounced, reducing the likelihood of reproductive suppression and resorption of foetuses (Munck *et al.*, 1984; Sapolsky, 2002; Young *et al.*, 2006). Related female house mice have previously been shown to have higher lifetime reproductive success in the communal nest compared to unrelated females (Konig, 1994b). In natural populations of wild house mice, females are more likely to nest with related partners, however if nest sites become crowded then females need to disperse and find a suitable nest site, or alternatively queue for reproductive opportunities in the natal nest and help to rear related pups (Hurst, 1987; Konig, 1994a). When females disperse they are likely to encounter a number of individuals of mixed parentage, some of which may be unrelated to themselves (see Rusu & Krackow, 2004; Weidt *et al.*, 2008). MUPs are known to be important for inbreeding avoidance (Sherborne *et al.*, 2007), and there was also recent evidence to suggest that a combination of MUPs and the major histocompatibility complex (MHC) could be important for social partner choice in house mice (Holmes, 2012). Females may therefore use MUPs to choose suitable social partner to nest with, which may then influence reproductive success, particularly for less competitive females (as suggested by the results found in this experiment).

6.5.4 Subsequent reproductive success of offspring born in competitive conditions

The results of this experiment provide little evidence to suggest that more competitive females have a reproductive advantage in the communal nest; although this assumption is based on a single reproductive event following competitive experience. Reproductive advantages of a mother's competitive status however, appear to be passed onto offspring, as daughters of more competitive females produced larger litters (on average) over three successive reproductive events. Competitive rank of mothers did not influence sex ratio of daughter's litters, but females reared in communal nests were themselves more likely to produce male biased litters. This is particularly interesting as females in this experiment did not significantly alter sex ratio of their litters from solitary to communal conditions and therefore it is unlikely to be an effect of litter order. Previous studies of reproductive success in house mice have used data spanning approximately six months, which is suggested to be an average life span for wild house mice (e.g. Konig, 1994a; Manning *et al.*, 1995). A prolonged study of reproductive success in competitively paired house mice may therefore highlight differences in reproductive success of more and less competitive females.

6.6 Conclusion

Reproductive output between communally nesting house mice appeared to be negatively influenced by female competition prior to and potentially throughout gestation. Although a higher frequency of more competitive females gave birth, there was little evidence to suggest that they gained reproductive advantages over their partners at the time of weaning. MUP profile similarity between female pairs positively influenced reproductive output, particularly for less competitive females. Some females failed to give birth in the communal nest, while other females lost entire or partial litters shortly after birth. However, there was no difference in the time female partners spent performing maternal behaviours, even if one female failed to give birth, suggesting that females may be motivated to care for young. There was also evidence to suggest that competitive rank of mothers can positively influence reproductive success of daughters, and that early nest environments influence sex ratio of litters at sexual maturity. The reproductive consequences of competition could be highly detrimental to females over their lifetime and

therefore it is important for females to adopt strategies that maximise opportunities to mate and improve the quality of their offspring born in order to obtain future fitness benefits.

Chapter 7 Competition in cooperative and communally caring species: effects on reproductive and life history traits

7.1 Chapter overview

The potential for competition between females or their offspring could be increased in species with cooperative or communal care of young, despite high levels of cooperation between closely related individuals. Cooperative species can be defined as those where a proportion of females do not regularly breed, but contribute to offspring care (i.e. those with a singular care system), whereas communal breeders are those species in which most/all adult females regularly breed and may provide care for offspring other than their own (i.e. those with a plural care system). Female reproductive success is typically skewed in species exhibiting cooperative care as the majority of females within social groups are non-breeding and help to rear the offspring of the dominant female(s). Female competition for breeding opportunities should therefore be high; litter size may also be increased, although competition between offspring may be relaxed if high levels of care are provided by helpers. By contrast, a higher proportion of females typically gain reproductive opportunities in species exhibiting communal care, where females may engage in communal nursing of one another's young. Here female competition for breeding opportunities may be less intense but there is potential for increased competition for investment between pups in the communal nest. Conflict between females over relative investment in young might also select for reduced investment in lactation in both communal and cooperative breeders, and competition between pups could lead to reduced post natal growth in communal breeding species. As a consequence of conflict and competition in these systems, there is therefore potential for selection to influence a broad range of reproductive and life history traits. Typically, sexual size dimorphism in mammals is male biased due to intra-male competition for mating opportunities. However if selection also acts to increase female body size as a result of increased competition, then the degree of sexual size dimorphism should be reduced in cooperative breeders. This effect may vary between cooperative and communal systems due to the differing degrees of potential conflict. Similarly if competition between pups is more intense in communal rearing systems, selection may favour increased growth rates in-utero or the production of larger neonates. Energy invested in scramble competition might also impact on offspring growth rates, despite increased investment available from communal rearing. In this chapter I

conduct phylogenetically-controlled comparative analyses to explore these hypotheses. I also examine other reproductive and life history traits to determine if they are affected due to the potential conflict in these systems, including components of milk quality and lactation, as well as measures of female reproductive output and offspring development. Contrary to expectations, I report evidence of reduced sexual size dimorphism in species with communal care of offspring rather than in species with cooperative care. Offspring of communal breeders also show some evidence of increased offspring growth rates in-utero, as predicted under increased competition, and reach age of independence later than other species. By contrast successfully reproducing females in cooperatively breeding species show evidence of increased reproductive output, with larger litters and shorter inter-birth intervals. Females of cooperatively breeding species also appear to reduce their investment in lactation, with a shorter duration of lactation and reduced milk protein content at peak lactation. Ecological conditions also influence life history traits in both cooperative and communal species, providing further evidence that cooperative breeders are more likely to be found in harsh climates. These results suggest that competition within cooperative and communally caring species has important consequences for the evolution of life history traits.

7.2 Introduction

Cooperatively breeding species are suggested to have evolved from socially monogamous mammalian species, leading to high levels of average kinship between group members. Non-breeding females may therefore maximise their inclusive fitness by helping to rear offspring of breeding relatives (Dugatkin, 1997; Hamilton, 1964a; Hamilton, 1964b), and consequently litter size of breeding females is likely to increase. In birds, cooperative breeding is more common among altricial species which require prolonged post natal care (Ligon & Burt, 2004). It also increases in species residing in harsher climates, which increases the potential for cooperative behaviour by helpers due to the high costs associated with dispersal (Jetz & Rubenstein, 2011). A study by Lukas and Clutton-Brock (2012b) showed that polytocy was a precondition for the evolution of cooperative breeding in monogamous mammals; therefore mating systems are also likely to influence the amount of competition between females for reproductive opportunities. Breeding females are assisted in protecting and feeding their young by helpers that are reproductively suppressed (Packer *et al.*, 1995; Young & Clutton-Brock, 2006), but helpers also gain

fitness benefits as a result of cooperative foraging, feeding, group defence and thermoregulation (Cockburn, 1998; Hayes, 2000; Lewis & Pusey, 1997).

Communal care is rare among mammalian species (Gilchrist, 2007), although it occurs in a wide range of taxa including some primate, rodent and carnivore species (Lewis & Pusey, 1997). Communally breeding species are likely to have evolved from plural breeding ancestors with polygynous mating systems (Lukas & Clutton-Brock, 2012a). Social groups that exhibit communal care tend to have low reproductive skew as most females reproduce, often with synchronised births (Sayler & Salmon, 1971). Combined offspring are pooled and provisioning is shared between females within the social group. However, there can also be high potential for conflict between females if reproductive opportunities and related resources are relatively scarce, but particularly between offspring that need to compete with siblings and other littermates (Hayes, 2000; Hodge *et al.*, 2007).

Both systems are of particular evolutionary interest due to the potential fitness costs associated with forgoing reproduction and/or caring for non-offspring, particularly during the lactation period when energetic demand peaks (Konig, 2006).

7.2.1 Sexual size dimorphism

As a consequence of increased competition for reproductive opportunities between females in cooperative systems, the intensity for selection to act on females is potentially increased, enhancing traits that improve a female's reproductive success either directly or indirectly (Clutton-Brock, 2009b; Rubenstein & Lovette, 2009). Typically in mammalian species, males are larger than females, with body mass playing an important role in intra-male competition (Trivers, 1972). However there are a number of examples of female biased sexual dimorphism in species of bats, mongooses and hyenas, some of which are cooperative breeders (Ralls, 1976). Dominant females may have increased body mass compared to other females within the group (Clutton-Brock *et al.*, 2006) and may show evidence of masculinisation with relatively high levels of testosterone (Drea, 2009), or enlarged genitalia (Glickman *et al.*, 1998). A recent comparative study on African starlings (*Sturnidae sp.*) showed evidence for reduced sexual dimorphism in terms of plumage and body size among cooperative breeders; this effect was thought to be the result of increased competition between females (Rubenstein & Lovette, 2009). However there is likely to be a trade-off associated with the costs of maintaining secondary sexual traits (Clutton-Brock

et al., 2006), particularly when combined with the energetic demand of reproduction in females and costs associated with lactation (Speakman, 2008). Within cooperatively caring species, the majority of non-reproducing females remain reproductively suppressed throughout their life and therefore gain fitness benefits by caring for related offspring. However selection may favour strategies that increase the likelihood of subordinate helpers obtaining dominant status, which may be related to body mass and age (Hodge *et al.*, 2008). Selection may therefore favour increased body mass in species where competition between females is potentially high, resulting in a decrease in sexual size dimorphism. It is also important to consider the selective force on male body mass as this will influence the degree of dimorphism. In cooperatively breeding species for example, female competition is likely to be relatively high due to low reproductive skew. However as it has been recently shown that cooperative breeding evolved from monogamous lineages we could expect selection for large male body mass to be relaxed as he needs only to defend a single female from rival males (Lukas & Clutton-Brock, 2012a). Conversely communally breeding species are thought to have evolved from polygynous ancestors where selection for relatively large male body mass is likely to be increased (Lukas & Clutton-Brock, 2012a). As reproductive skew is much lower in communal systems we could expect competition between females to be relatively lower than that found in cooperative systems and therefore selection for increased body mass to be reduced, however evidence in the literature and found in this thesis (Chapter 3) suggests otherwise.

7.2.2 Effects on reproductive output

Species that cooperatively or communally rear young are suggested to have increased reproductive output and give birth to larger litters than if they bred alone (Clutton-Brock, 2002; Gilchrist, 2007; Jennions & Macdonald, 1994; Komdeur *et al.*, 2001; Konig, 1993; Lukas & Clutton-Brock, 2012b), particularly where the costs of rearing young are high or ecological conditions are harsh (Lukas & Clutton-Brock, 2012b). Females that receive care from helpers may have shorter inter-birth intervals (Mitani & Watts, 1997; Ross & Maclarnon, 2000) and litter sizes may be increased where group members can be reproductively suppressed (Moehlman & Hofer, 1997). Although kin selection is thought to play an important role in the evolution and maintenance of cooperative breeding (Dugatkin, 1997; Hamilton, 1964a; Hamilton, 1964b), there are examples where unrelated individuals contribute to offspring care (Clutton-Brock, 2002; Clutton-Brock, 2009a;

Clutton-Brock *et al.*, 2000). The presence of unrelated group members may therefore further increase the potential for competition for reproductive opportunities in both communal (see Chapters 1 and 5) and cooperative breeders (West *et al.*, 2002), as well as affect the quality of help that is provided, which could impact on offspring growth (Kokko *et al.*, 2002; Wright *et al.*, 2009).

7.2.3 Offspring competition

Sibling competition has been extensively studied in avian species, as small brood size and infrequent, unpredictable provisioning of young is associated with nestling aggression and contest competition (see Drummond, 2001). Over the past fifteen years however, there have been increasing numbers of studies of sibling competition among mammal species, providing contrasts between two different feeding systems (e.g. Hudson & Trillmich, 2008). Unlike birds, many mammals adopt relatively immobile nursing postures while their offspring jostle for position; therefore there is little opportunity for mothers to be selective over the offspring nursed (Hudson & Trillmich, 2008). Individuals that are larger at birth tend to have advantages against littermates in competition for milk (Hodge *et al.*, 2009; Mock & Parker, 1997), and in species where males are the dominant sex, males can outcompete sisters for access to food (e.g. Bonisoli-Alquati *et al.*, 2011). Although there are likely to be two nipples available for every dependent offspring, (Gilbert, 1986), females may restrict access to nipples by lying on their sides while nursing (e.g. Fraser *et al.*, 1995). Once offspring attach to a nipple, they usually remain there until it is depleted (Cramer & Blass, 1983), meaning that unsuccessful offspring are unlikely to nurse from the same nipple successively (Cramer & Blass, 1983). Certain mammae may be also be more productive, resulting in increased weight gain for offspring that can obtain priority access to them (Fraser, 1990; Mock & Parker, 1997). Therefore the potential for high levels of sibling competition can occur, even when the number of nipples is greater than dependent offspring (Mock & Parker, 1997; Stockley & Parker, 2002).

In a study of carnivore and insectivore species, Stockley and Parker (2002) suggested that pre natal growth rates (relative to maternal body size) increase when post natal sibling competition is high. However, the increase in energetic costs associated with producing larger offspring at birth could result in parent-offspring conflict, which may lead to selection for shorter gestation length but increased pre natal growth rates (Stockley & Parker, 2002; Trivers, 1974). Reductions in gestation length have consequences on

offspring development; this is observed in mammals that produce altricial young (Martin & Maclarnon, 1985; Stockley & Parker, 2002). In communally breeding species, competition between offspring could be intensified, as there are larger numbers of dependent young competing for opportunities to feed. Competition could be further increased if the combined offspring were also unrelated to each other. Consequently, there could be selection for increased pre natal growth in communally breeding species to provide competitive advantages in terms of body mass/size (Stockley & Parker, 2002). Post natal growth however may be negatively affected if offspring are using energy in scramble competition. In cooperatively breeding species, competition between females is likely to be increased due to high reproductive skew. Litter size may therefore be increased, but competition between offspring could be relaxed due to the presence of many helpers (Hodge, 2005). Consequently, selection may favour shorter gestation lengths and shorter inter-birth intervals in cooperatively breeding species.

7.2.4 Effects on lactation

As many communal species exhibit non-offspring nursing, energetic demand and conflict between females is likely to be increased as there are more offspring to care for (Konig, 1993; Konig, 2006). Communal nursing may have evolved to enable females to share the costs of lactation and to increase the period of time between nursing bouts (Konig, 2006; Manning *et al.*, 1995). However, even under standard conditions of nursing, females lose weight during the lactation period and may also undergo physiological changes in the liver, kidneys and digestive tract (Speakman, 2008). Offspring are entirely dependent on milk throughout the first stage of lactation and therefore nutrients essential to growth are gained from the milk of lactating females (Langer, 2008). The main energy source of milk generally consists of fats, which are increased in quantity during peak lactation (Konig, 2006). However when litter size is increased, females are unable to increase the quality of their milk proportionally over the lactation period (Landete-Castillejos *et al.*, 2005). As a consequence larger litters grow more slowly and have a lower overall weaning weight (Konig *et al.*, 1988). It could therefore be predicted that communally caring females may have lower quality milk than other polytocous species, due to the increase in demand by multiple litters. As lactational demand is likely to increase for communally breeding species, it is possible that females may attempt to reduce lactation length to reduce energetic demand (Fuchs, 1982). In cooperatively breeding species with high reproductive

skew, the demand of milk is not likely to be as high as observed in plurally breeding species. The presence of helpers in the nest may enable breeding females to reduce investment in their offspring post-natally, and therefore there may be selection for shorter lactation length.

7.2.5 Ecological factors

Ecological conditions have been suggested as a driving force for the evolution of cooperative breeding as many species are thought to live in relatively harsh conditions (Lukas & Clutton-Brock, 2012a). The habitat saturation hypothesis states that under limiting food and/or space, individuals are more likely to cooperate (Getz *et al.*, 1992) and individuals are also less likely to disperse when habitat quality is poor, due to the risk of not finding a suitable nest or burrow (Koenig *et al.*, 1992). Jetz and Rubenstein (2011) found that cooperative care was more common amongst bird species living in habitats with low annual rainfall. However, as a consequence of living in challenging conditions, nest sites could become crowded and there would be increased competition for resources (Hayes, 2000). Therefore ecological factors are also likely to be important when studying life history traits in mammalian cooperative breeders.

7.2.6 Comparative study aims

As outlined above, several lines of evidence suggest that competition could influence reproductive and life history traits amongst cooperative and communally caring species. Comparative methods have been increasingly used to examine the lineages under which cooperative breeding evolved (Lukas & Clutton-Brock, 2012a; Lukas & Clutton-Brock, 2012b), and also to establish the conditions under which communal nursing occurs (Packer *et al.*, 1992). However there has yet to be a study that investigates how the potential for competition may have affected life history traits in both cooperative and communally caring species. In this chapter I therefore conduct phylogenetically-controlled comparative analyses using data collected on a variety of reproductive and life history traits that may be affected by conflict between females and/or offspring. I first assess whether mammalian species with cooperative and/or communal care have reduced sexual size dimorphism compared to other species, as predicted due to the increased potential for intra-sexual competition between females. I also consider whether cooperative and/or communally breeding animals have increased litter size compared to other polytocous species, and if

offspring development is enhanced by growth rates in-utero for communally breeding species, as predicted due to increased potential for competition between offspring. As there may be increased conflict over relative investment in young, I investigate if investment in lactation is reduced for both cooperative and communal species and if post natal growth is reduced in communal species due to increased competition between pups.

7.3 Methods

7.3.1 Data Collection

Information on the occurrence of cooperative and communal care systems was collected from a variety of published reviews and from a systematic search of the available literature on social systems (Ebensperger & Hayes, 2008; Hayes, 2000; Lewis & Pusey, 1997; Lukas & Clutton-Brock, 2012a; Riedman, 1982; Rowe, 1996). Cooperative species were defined as those where a proportion of females do not regularly breed, but contribute to offspring care (i.e. those with a singular care system). Communal breeders were defined as species where most/all adult females regularly breed and may provide care for offspring other than their own (i.e. those with a plural care system). Data from captive studies was excluded due to the potential constraints of housing conditions and breeding programmes.

Data for reproductive rate (litter size at birth, inter-birth interval), offspring development (natal mass, weaning mass, age at independence, age at sexual maturity), lactation parameters (lactation length, milk fat content, milk protein content) and body mass were all obtained using a number of published sources, but primarily from the PanTHERIA database (Jones *et al.*, 2009). The PanTHERIA data set contains 100,740 lines of biological data for extant and recently extinct mammalian species collected over a period of 3 years by 20 individuals. All of the data collected by Jones *et al* (2009) uses strict criteria to ensure there was no duplication when using primary and secondary sources, and that each variable was calculated appropriately to result in a single value for each species. Outliers were also identified and either carefully corrected or excluded (Jones *et al.*, 2009). Pre natal offspring growth rates were calculated by dividing natal mass by gestation length. Post natal offspring growth rates were calculated by subtracting natal mass from weaning mass, and dividing by the age in which offspring reach independence. Milk composition data such as fat and protein content were collected from a number of published sources (Barton & Capellini, 2011; Langer, 2008; Riek, 2011). Ecological data such as average home range, annual precipitation rate and average temperature were also collected from PanTHERIA and other published reviews (Swihart *et al.*, 1988). Sexual size dimorphism was calculated as the difference in male and female body mass, relative to male body mass (as in Rubenstein & Lovette, 2009). Species missing from this data source were identified in other published sources and using averages when multiple values were available

(Hayssen & van Tienhoven, 1993; Nowak & Wilson, 1999). Missing data across the data set meant that sample sizes were sometimes reduced.

All continuous life history variables were log-transformed prior to analysis to match assumptions of a normal distribution, with the exception of milk composition data which was proportional and therefore transformed using the arsine square root method. Ecological data met parametric assumptions and therefore was left untransformed. Species that were described as neither cooperative nor communal were either monotocous or polytocous, resulting in the variable 'litter size at birth' to be binomially distributed. In order to normalise the data I therefore removed all monotocous species from the analysis. In a recent study, Lukas and Clutton-Brock (2012b) found that cooperative care evolved in lineages with polytocous females, therefore by limiting the data set to only polytocous species I was able to make a direct comparison between care systems without the potentially confounding effects of litter size and precociality in monotocous species. A condensed data set and relevant references can be found in the Appendix of this thesis.

7.3.2 Comparative methods

Comparative methods are used to uncover patterns of correlated evolution between traits, enabling common selective pressures to be established (Butler & King, 2004; Harvey & Pagel, 1991; Nunn, 2011). However, direct comparison of data on extant species may violate the assumption of independence necessary for regression analysis as closely related species can often share traits due to shared common ancestry (Felsenstein, 1985). Phylogenetic methods require an estimate of phylogenetic relationships between species included in the data set, ideally including branch length information. Therefore in this analysis phylogenetic reconstruction was conducted using information from the recently published mammalian super tree with dated branch lengths (Bininda-Emonds *et al.*, 2007), which was pruned to match the species present in the data set using the APE package (Paradis *et al.*, 2004) in the statistical software R v. 2.15.1 (R Development Core Team, 2010). All species names where matched from the data set to the tree according to 'Mammal Species of the World' (Wilson & Reeder, 1993; Wilson & Reeder, 2005).

As closely related species share a large degree of common ancestry, and are therefore more likely to share similar traits, I used a Phylogenetic-Generalised Least Squares (PGLS) approach to control for non-independence. Briefly this method employs a maximum

likelihood (ML) framework to estimate an index of phylogenetic dependence, Pagel's lambda (λ), based on the extent to which shared ancestry explains the data (Freckleton *et al.*, 2002; Pagel, 1999). A Brownian method of character evolution is assumed (i.e. the degree of change in a character between 2 species is proportional to the time since they diverged) (Felsenstein, 1985). When $\lambda = 0$ the trait is not related to the phylogeny and branch lengths are altered to all become the same length, however when $\lambda = 1$, independent contrast methods can be used since the data fits a Brownian model and the branch lengths are unaltered. When λ falls between 0 and 1, internal branches are reduced as the phylogenetic signal in the trait is not as pronounced as under the Brownian model. Therefore this approach estimates the most likely transformation of branch lengths according to λ and is conducted using the CAPER package in R (Orme *et al.*, 2011).

Before running the analysis, all data variables were checked to see if the assumptions of linear modelling were met. Model diagnostic plots were used to establish if the data were normally distributed following appropriate transformations of the data set (see Section 7.3.1). A series of models were then constructed to establish whether cooperative or communally caring species differed from other species in reproductive rate, offspring development, lactation parameters and sexual size dimorphism. In most of the models, female body mass was entered as a covariate due to the potential effects on reproductive output and offspring size. Litter size was added to most models due to the potential effects on offspring development. Where data for average temperature and precipitation were available, they were added to models investigating reproductive rate, lactation and offspring development as these may be affected by seasonality. However due to missing data, ecological data could not be included in all relevant analyses. I also removed data according to the system being tested i.e. when constructing the models to investigate cooperative species, all communal species data were removed from the analysis, and when communal species were examined all data points for cooperative species were removed. A summary of all of the results from the PGLS models for cooperative and communal species can be found in Table 7.1 and Table 7.2 respectively.

7.4 Results

Data were collected for 509 mammalian species across a range of life history traits. Thirty-four species were identified as cooperative breeders (i.e. a singular care system where one female generally monopolises reproduction and non-reproducing individuals help to rear the young and defend the nest/burrow). Fifty-eight species were identified as communally caring species (i.e. plural care systems where two or more females reproduce and rear their young in the same nest/burrow). A total of 417 species were identified as being neither cooperative nor communal breeders.

7.4.1 Cooperatively caring species

7.4.1.1 Sexual size dimorphism

Despite the potential for conflict between females in cooperative species there was no evidence that sexual size dimorphism was reduced relative to non-cooperatively breeding species ($t = -0.496$, $df = 3, 158$, $p = 0.621$; Table 7.1a).

7.4.1.2 Reproductive output

There is a strong positive association between the presence of cooperative care and reproductive output after control for phylogeny, female body mass and ecological factors (Table 7.1b). Compared to non-cooperative species, cooperative breeders produce significantly larger litters ($t = 2.565$, $df = 5, 163$, $p = 0.011$) with a negative influence of female body mass ($t = -2.235$, $df = 5, 163$, $p = 0.027$), and have shorter inter-birth intervals ($t = -2.304$, $df = 4, 89$, $p = 0.024$). Ecological factors were also important as average temperature negatively influenced litter size ($t = -4.316$, $df = 5, 163$, $p < 0.001$) and there was a non-significant negative effect of average precipitation rates on litter size ($t = -1.893$, $df = 5, 163$, $p = 0.060$). Gestation length was significantly longer in cooperatively breeding species compared to other polytocous species ($t = 2.352$, $df = 4, 145$, $p = 0.020$) and there was also a non-significant trend for increased oestrus cycle length in cooperative species ($t = 1.936$, $df = 4, 13$, $p = 0.075$). Average temperature explained some of the variance shown in oestrus cycle length among polytocous species (cooperative $t = 6.321$, $df = 4, 13$, $p < 0.001$) as did female body mass ($t = 6.702$, $df = 4, 13$, $p < 0.001$).

7.4.1.3 Lactation

Females of cooperatively caring species have shorter lactation lengths than other polytocous species (t = -2.958, df = 6, 59, p = 0.004; Table 7.1c). Milk protein content is also significantly lower in cooperative species compared to non-cooperative species (t = -2.621, df = 6, 11, p = 0.024; Table 7.1c), despite litter size at birth positively influencing the amount of protein produced (t = 2.716, df = 6, 11, p = 0.020; Table 7.1c). However fat content of milk is not significantly different between cooperative breeders and other polytocous species (t = -0.972, df = 6, 11, p = 0.352; Table 7.1c).

7.4.1.4 Offspring development

There is no evidence that cooperative care affects offspring development in polytocous species (Table 7.1d). The age at which offspring of cooperative breeders reach independence and sexual maturity is not significantly different to other polytocous species (independence t = 0.521, df = 6, 82, p = 0.603; maturity t = -0.721, df = 6, 109, p = 0.472), although average temperature negatively influences the age that offspring reach sexual maturity (t = -2.859, df = 6, 109, p = 0.005). Despite the increase in gestation length, offspring growth in-utero was not found to differ from that of other polytocous species (t = -0.625, df = 4, 122, p = 0.533), and there is no difference in daily weight gain between offspring of cooperative breeders and other polytocous mammals (t = -0.134, df = 6, 53, p = 0.894).

7.4.2 Communally caring species

7.4.2.1 Sexual size dimorphism

After controlling for phylogeny and mating system, species with communal care of offspring have significantly reduced sexual size dimorphism relative to non-communally breeding species (t = -2.024, df = 3, 166, p = 0.045; Table 7.2a).

7.4.2.2 Reproductive output

In contrast to cooperative species there is no evidence of increased reproductive output in species with communal care (Table 7.2b). Litter size at birth is not larger in communally caring species compared to other polytocous species (t = -0.526, df = 5, 170, p = 0.599), although both female body mass and average temperature negatively influence litter size

(body mass t = -2.261, df = 5, 170, p = 0.025; temperature t = -5.324, df = 5, 170, p <0.001). Communally caring females do not have significantly different inter-birth intervals (t = -0.521, df = 5, 94, p = 0.603) or gestation periods (t = -0.822, df = 4, 154, p = 0.412) compared to non-cooperative females, but oestrus cycle length is significantly longer (t = 2.840, df = 4, 19, p = 0.010). Average temperature appears to positively influence both the number of litters born per year (t = 3.210, df = 5, 84, p = 0.002) and oestrus cycle lengths (t = 3.450, df = 4, 19, p = 0.003).

7.4.2.3 Lactation

There was no evidence that communal care influences lactation parameters (Table 7.2c). Communally nesting females have similar lactation lengths to other polytocous species (t = 0.682, df = 6, 67, p = 0.498) and there was no evidence that the quality of milk was altered in communal species (fat t = -0.550, df = 6, 12, p = 0.592; protein t = 1.084, df = 6, 12, p = 0.300). Litter size at birth was however found to positively influence milk protein content (t = 2.765, df = 6, 12, p = 0.017).

7.4.2.4 Offspring development

After controlling for body mass, average temperature, litter size and gestation length (linked to relative altriciality of young), the comparative analysis indicated that the offspring of communal breeders reach independence later than other species (t = 2.454, df = 6, 89, p = 0.016; Table 7.2d) and there was no evidence that communal breeders have enhanced offspring development. Age at sexual maturity did not differ between offspring born in communal nests or other polytocous species (t = -1.343, df = 6, 120, p = 0.182; Table 7.2d), although average temperature negatively influenced this trait (t = -1.343, df = 6, 120, p = 0.002; Table 7.2d). Offspring growth is not significantly greater in communally caring species compared to other polytocous species in terms of growth rate per day from birth to weaning (t = -0.495, df = 6, 65, p = 0.622; Table 7.2d). There was however a non-significant trend for offspring of communal breeders to gain more weight in-utero (t = 1.804, df = 5, 133, p = 0.074; Table 7.2d).

Table 7.1 - Phylogenetic generalised linear model analysis (PGLS) results comparing cooperatively caring species and other polytocous mammals.
A series of models were constructed to establish whether cooperatively caring species differ from other polytocous species (excluding communally breeding species) in sexual size dimorphism (a), reproductive output (b), lactation parameters (c), and offspring development (d). A number of life history variables were included if they influenced the trait of interest (e.g. female body mass on reproductive output). Ecological data (average monthly temperature and precipitation) were collected and included where seasonality may affect the trait of interest. Mating system (i.e. monogamous or other) was included as a dichotomous variable for body size dimorphism model due to the potential influence of competition between females. Care system (i.e. cooperative or other) was included as dichotomous variables for each of the models.

Traits			ML λ	df	Variables	Estimate ± SE	t-value	p-value
a)	**Body size dimorphism**	**Body mass dimorphism**	0.000	3, 158	Mating system	0.032 ± 0.019	1.654	0.100
					Care system (cooperative)	**-0.014 ± 0.028**	**-0.496**	**0.621**
b)	**Reproductive rate**	**Litter size (birth)**	0.926	5, 163	Female body mass	-0.053 ± 0.024	-2.235	0.027*
					Temperature (^{o}C)	-0.001 ± 0.000	-4.316	<0.001***
					Precipitation (mm)	-0.000 ± 0.000	-1.893	0.060
					Care system (cooperative)	**0.082 ± 0.032**	**2.565**	**0.011***
		Offspring mass (birth)	0.962	5, 125	Female body mass	0.624 ± 0.036	17.284	<0.001***
					Litter size birth	-0.353 ± 0.113	-3.124	0.002**
					Gestation length	0.519 ± 0.194	2.678	0.008**
					Care system (cooperative)	**0.012 ± 0.041**	**-0.289**	**0.773**
		Inter-birth interval	0.977	4, 89	Female body mass	0.197 ± 0.041	4.830	<0.001***
					Temperature (^{o}C)	0.000 ± 0.000	-0.050	0.961
					Care system (cooperative)	**-0.088 ± 0.038**	**-2.304**	**0.024***
		Gestation length	1.000	4, 145	Female body mass	0.071 ± 0.014	5.225	<0.001***
					Litter size birth	-0.187 ± 0.038	-4.848	<0.001***
					Care system (cooperative)	**0.028 ± 0.012**	**2.352**	**0.020***
		Oestrus cycle length	0.000	4, 13	Female body mass	0.171 ± 0.026	6.702	<0.001***
					Temperature (^{o}C)	0.002 ± 0.000	6.321	<0.001***
					Care system (cooperative)	**0.108 ± 0.056**	**1.936**	**0.075**

Traits		ML λ	df	Variables	Estimate ± SE	t-value	p-value
c) Lactation	**Lactation length**	0.858	6, 59	Female body mass	0.125 ± 0.048	2.609	0.011*
				Litter size birth	-0.029 ± 0.155	-1.901	0.062
				Gestation length	0.441 ± 0.248	1.778	0.081
				Temperature (^{o}C)	0.000 ± 0.000	1.312	0.195
				Care system (cooperative)	**-0.158 ± 0.053**	**-2.958**	**0.004***
	Milk fat content	0.000	6, 11	Female body mass	-3.566 ± 4.506	-0.0791	0.445
				Litter size birth	24.988 ± 19.199	1.302	0.220
				Lactation length	0.749 ± 11.063	0.068	0.947
				Temperature (^{o}C)	-0.027 ± 0.031	-0.864	0.406
				Care system (cooperative)	**-7.385 ± 7.598**	**-0.972**	**0.352**
	Milk protein content	0.000	6, 11	Female body mass	-0.591 ± 2.916	-0.203	0.843
				Litter size birth	33.741 ± 12.423	2.716	0.020*
				Lactation length	-4.086 ± 7.159	-0.571	0.580
				Temperature (^{o}C)	-0.009 ± 0.020	-0.434	0.673
				Care system (cooperative)	**-12.887 ± 4.917**	**-2.621**	**0.024***

Traits			ML λ	df	Variables	Estimate ± SE	t-value	p-value
d)	**Offspring development**	**Offspring growth rate (day^{-1})**	0.850	6, 53	Female body mass	0.564 ± 0.072	7.819	<0.001***
					Litter size birth	-0.043 ± 0.260	-0.165	0.870
					Gestation length	-0.280 ± 0.285	-0.981	0.331
					Temperature (^oC)	-0.001 ± 0.001	-1.593	0.117
					Care system (cooperative)	**-0.017 ± 0.126**	**-0.134**	**0.894**
		Offspring growth rate in-utero	0.951	5, 121	Female body mass	0.619 ± 0.036	17.122	<0.001***
					Litter size birth	-0.315 ± 0.116	-2.713	0.008**
					Gestation length	-0.473 ± 0.191	-2.476	0.015*
					Care system (cooperative)	**-0.025 ± 0.040**	**-0.625**	**0.533**
		Age at independence	0.829	6, 82	Female body mass	0.234 ± 0.052	4.505	<0.001***
					Litter size birth	-0.104 ± 0.181	-0.578	0.565
					Gestation length	0.538 ± 0.239	2.252	0.027*
					Temperature (^oC)	-0.000 ± 0.000	-0.490	0.625
					Care system (cooperative)	**0.055 ± 0.105**	**0.521**	**0.604**
		Age at sexual maturity	0.912	6, 109	Female body mass	0.155 ± 0.045	3.438	0.001***
					Litter size birth	-0.056 ± 0.156	-0.360	0.720
					Gestation length	0.396 ± 0.232	1.702	0.092
					Temperature (^oC)	-0.001 ± 0.000	-2.859	0.005**
					Care system (cooperative)	**-0.042 ± 0.058**	**-0.721**	**0.472**

Table 7.2 - Phylogenetic generalised linear model analysis (PGLS) results comparing communally caring species and other polytocous mammals.
A series of models were constructed to establish whether communally caring species differed from other polytocous species (excluding cooperatively breeding species) in sexual size dimorphism (a), reproductive output (b), lactation parameters (c), and offspring development (d). A number of life history variables were included if they influenced the trait of interest (e.g. female body mass on reproductive output). Ecological data (average monthly temperature and precipitation) were collected and included where seasonality may affect the trait of interest. Mating system (i.e. monogamous or other) was included as a dichotomous variable for body size dimorphism model due to the potential influence of competition between females. Care system (i.e. communal or other) was included as dichotomous variables for each of the models.

Traits			ML λ	df	Variables	Estimate ± SE	t-value	p-value
a)	**Body size dimorphism**	**Body mass dimorphism**	0.000	3, 166	Mating system	0.027 ± 0.026	1.037	0.301
					Care system (communal)	**-0.059 ± 0.029**	**-2.024**	**0.045***
b)	**Reproductive rate**	**Litter size (birth)**	0.935	5, 170	Female body mass	-0.051 ± 0.023	-2.261	0.025*
					Temperature (^{o}C)	-0.001 ± 0.000	-5.324	<0.001***
					Precipitation (mm)	-0.000 ± 0.000	-1.128	0.261
					Care system (communal)	**-0.012 ± 0.023**	**-0.526**	**0.599**
		Offspring mass (birth)	0.938	5, 136	Female body mass	0.602 ± 0.036	16.570	<0.001***
					Litter size birth	-0.216 ±0.121	-1.787	0.076
					Gestation length	0.725 ± 0.192	3.777	<0.001***
					Care system (communal)	**0.023 ± 0.033**	**0.709**	**0.479**
		Inter-birth interval	0.996	5, 94	Female body mass	0.147 ± 0.039	3.749	<0.001***
					Litter size birth	-0.241 ± 0.165	-1.456	0.149
					Temperature (^{o}C)	-0.001 ± 0.000	-1.615	0.110
					Care system (communal)	**-0.017 ± 0.032**	**-0.521**	**0.603**
		Gestation length	1.000	4, 154	Female body mass	0.060 ± 0.014	4.234	<0.001***
					Litter size birth	-0.176 ± 0.041	-4.293	<0.001***
					Care system (communal)	**-0.009 ± 0.010**	**-0.822**	**0.412**
		Oestrus cycle length	0.000	4, 19	Female body mass	0.160 ± 0.041	3.898	0.001***
					Temperature (^{o}C)	0.002 ± 0.001	3.450	0.003**
					Care system (communal)	**0.223 ± 0.079**	**2.840**	**0.010****

Traits		ML λ	df	Variables	Estimate ± SE	t-value	p-value
c) Lactation	**Lactation length**	0.953	6, 67	Female body mass	0.143 ± 0.046	3.106	0.003**
				Litter size birth	-0.021 ± 0.144	-0.143	0.887
				Gestation length	0.639 ± 0.248	2.575	0.012*
				Temperature (^{o}C)	0.001 ± 0.000	2.965	0.004**
				Care system (communal)	**0.023 ± 0.034**	**0.682**	**0.498**
	Milk fat content	0.000	6, 12	Female body mass	-2.842 ± 4.842	-0.587	0.568
				Litter size birth	26.771 ± 16.672	1.606	0.134
				Lactation length	2.179 ± 11.165	0.195	0.849
				Temperature (^{o}C)	0.012 ± 0.051	0.232	0.820
				Care system (communal)	**-3.027 ± 5.503**	**-0.550**	**0.592**
	Milk protein content	1.000	6, 12	Female body mass	2.947 ± 1.871	1.576	0.141
				Litter size birth	17.962 ± 6.496	2.765	0.017*
				Lactation length	-8.282 ± 5.509	-1.503	0.159
				Temperature (^{o}C)	0.042 ± 0.022	1.935	0.077
				Care system (communal)	**1.123 ± 1.036**	**1.084**	**0.300**

Traits		ML λ	df	Variables	Estimate ± SE	t-value	p-value
d) Offspring development	**Offspring growth rate (day^{-1})**	0.000	6, 65	Female body mass	0.405 ± 0.089	4.567	<0.001***
				Litter size birth	0.569 ± 0.348	1.635	0.107
				Gestation length	0.553 ± 0.355	1.560	0.124
				Temperature (^{o}C)	-0.001 ± 0.001	-1.446	0.153
				Care system (communal)	**-0.056 ± 0.113**	**-0.495**	**0.622**
	Offspring growth rate in-utero	0.834	5, 133	Female body mass	0.649 ± 0.047	13.755	<0.001***
				Litter size birth	-0.248 ± 0.164	-1.510	0.133
				Gestation length	-0.400 ± 0.232	-1.725	0.087
				Care system (communal)	**0.086 ± 0.048**	**1.804**	**0.074**
	Age at independence	0.958	6, 89	Female body mass	0.276 ± 0.062	4.426	<0.001***
				Litter size birth	0.002 ± 0.210	0.103	0.918
				Gestation length	0.842 ± 0.309	2.725	0.008**
				Temperature (^{o}C)	0.000 ± 0.000	0.190	0.850
				Care system (communal)	**0.145 ± 0.059**	**2.454**	**0.016***
	Age at sexual maturity	0.913	6, 120	Female body mass	0.171 ± 0.043	3.974	<0.001***
				Litter size birth	-0.244 ± 0.154	-1.580	0.117
				Gestation length	0.255 ± 0.224	1.136	0.258
				Temperature (^{o}C)	-0.001 ± 0.000	-3.120	0.002**
				Care system (communal)	**-0.053 ± 0.040**	**-1.343**	**0.182**

7.5 Discussion

The results of this analysis suggest that conflict in cooperative and communally caring species may influence certain reproductive and life history traits in mammals, although as discussed below, several findings were unexpected.

7.5.1 Sexual size dimorphism

Despite the high potential for competition between cooperatively breeding females, there is little evidence for a reduction in sexual size dimorphism in terms of body mass. High levels of relatedness in cooperative breeders could result in relatively limited competition, however this seems unlikely on the basis of previous evidence (Briga *et al.*, 2012; Cant *et al.*, 2002; Clutton-Brock, 2009a; Gilchrist, 2007; Hodge *et al.*, 2011; Hodge *et al.*, 2008; Russell *et al.*, 2004; Young *et al.*, 2006). Another possibility is that competition may take different forms and body size might not be the most important factor. However, morphological changes generally occur when a dominant female assumes her position, resulting in increased body mass compared to non-reproducing females; this has been observed in meerkats (Clutton-Brock *et al.*, 1998; Russell *et al.*, 2004) and naked mole rats (Faulkes & Abbott, 1997; Lacey & Sherman, 1997). Naked mole rats have particularly enhanced morphological change on obtaining breeding rank, not only gaining weight, but also show elongation of the lumbar vertebrae which is thought to be under hormonal control (O'Riain *et al.*, 2000). The data collected for this analysis contained average body mass for each species and therefore may not reflect the size of breeding females in cooperative systems, which may explain this finding.

Among communally caring species, body mass dimorphism is significantly reduced, which may be explained by selection acting on increased female body mass. Although competition is thought to be relatively low between females due to low reproductive skew, there remains potential for competition between females when gaining access to breeding males, or in order to acquire suitable nest sites/burrows or other important resources such as food. In my experimental work I have shown that body mass is an important predictor of competitive ability and reproductive success in female house mice (Chapters 3 and 6) and the influence of body mass on reproductive success and offspring development has also been demonstrated in other communally breeding species such as banded mongooses (*Mungos mungo*) (Hodge *et al.*, 2009). Low reproductive skew observed in communally

breeding species indicates that the majority of females are capable of breeding within a group, although the potential for competition for mates and resources are likely to be present throughout reproductive life. Although we could expect strong selection on increased male body mass due to communal breeding systems evolving from polygynous ancestors (Lukas & Clutton-Brock, 2012a), there must be strong selective pressure on increased body mass in females to result in a reduction in sexual size dimorphism. Through increased body mass, females are likely to gain a competitive advantage over female rivals when competing for resources necessary for reproduction. In addition to this, a reduction in sexual size dimorphism would also provide relatively larger females with a greater probability of protecting their offspring from intruding infanticidal males. Therefore by including measures of sexual size dimorphism rather than female body mass alone, I was able to examine the selective pressures on both sexes in the different breeding systems.

7.5.2 Reproductive output

The analyses of other life history traits confirmed that cooperatively breeding mammals produce larger litters and have shorter inter-birth intervals compared to other polytocous species. The presence of helpers in the nest is likely to be a driving factor, as mothers can reduce post natal investment in their young under these conditions and direct energy towards increasing reproductive output. Non-breeding individuals gain indirect benefits from caring for the offspring of the breeding female, due to high levels of relatedness between group members (Hamilton, 1964a; Hamilton, 1964b; Solomon & Getz, 1997), but breeding females are also suggested to coerce helpers through reproductive suppression and threats of eviction (Clutton-Brock *et al.*, 2001a; Saltzman, 2010; Young *et al.*, 2008). Communal species however did not appear to produce larger litters compared to other polytocous species, or produce litters at an increased rate. This may be a constraint of communal nursing as larger litter sizes would potentially increase lactational demand for each breeding female in the nest/burrow (Knight *et al.*, 1986; Konig *et al.*, 1988).

Oestrus cycle length is prolonged in communal species, which could be related to the care system or be a function of group living, although this was not tested here. Prolonged oestrus cycle lengths could provide more time to compete for opportunities to mate, particularly if cycles are synchronised and males are encountered simultaneously (Emlen & Oring, 1977). Relatively short oestrus cycles may therefore increase competition between females which could then have detrimental consequences for reproductive

success. In cooperative species, non-breeding individuals are not physiologically sterile as reproductive differences are thought to be predominantly maintained by aggressive interactions with the breeding female (Faulkes & Abbott, 1997; Lacey & Sherman, 1997). Subordinate meerkats (*Suricata suricatta*) have been observed to mate around the time that the dominant female gives birth, and are more likely to be successful if they are older and heavier (Clutton-Brock *et al.*, 2001a; Clutton-Brock *et al.*, 2008; Young *et al.*, 2008). However oestrus cycle length could be affected by the stress of subordination and directed aggression (Carlson *et al.*, 2004; Young *et al.*, 2006). This may result in prolonged periods of dioestrus which would increase overall cycle length. In this study there was a non-significant trend for prolonged oestrus cycle length in cooperatively breeding species, which may have been influenced if there are differences in cycle length between dominant breeders and subordinate helpers.

7.5.3 Offspring development

Due to the high potential for competition between littermates in communally breeding species, it is surprising to find no differences in gestation length and natal body mass compared to other polytocous species. There was however evidence that offspring growth in-utero is increased, suggesting that females invest more in their young during the pre natal stage compared to other polytocous species. Increased pre natal growth can provide offspring with competitive advantages in the communal nest (through increased body mass) when access to lactating females is high (Royle *et al.*, 1999; Stockley & Parker, 2002; Weber & Olsson, 2008); although there was no evidence of increased natal mass for communal species in this study. This suggests a cost of competition in-utero, as increased pre natal growth should result in increased natal mass. In addition, the age at which offspring reached independence within communal care systems was later (compared other polytocous species), which could be the result of increased scramble competition between littermates. Energy expended on competition during the lactation period could therefore result in slower development. Alternatively the cost of increasing lactational demand for females may affect milk quality, which would subsequently affect offspring growth (see below).

In cooperatively breeding species gestation length was increased in response to the increase in litter size, but natal mass was not significantly different to other polytocous species. This suggests that breeding females in cooperative systems invest more pre

natally, producing more numerous young, which would also increase the number of available helpers for future offspring (Jarvis, 1981; Koenig *et al.*, 1992). Larger litter size is generally associated with shorter gestation length and more altricial young (see Stockley & Parker, 2002), therefore periods of development are increased during the post natal stage. Cooperative females however do the opposite, and invest more in their young during a prolonged period of gestation. In cooperative species such as naked mole rats and meerkats, breeding females are provisioned by helpers (Lacey & Sherman, 1997), and therefore may be able to redirect the energy they would have spent on foraging back to their unborn young. Gestation may be energetically less costly than lactation and therefore breeding females may extend gestation and limit their investment in lactation.

7.5.4 Lactation

In agreement with the above hypothesis, cooperatively breeding species have shorter lactation lengths compared to other polytocous species when correcting for litter mass. Due to the presence of helpers, females can reduce the costs associated with nursing and decrease the interval in between births (Langer, 2008; Riek, 2011). Lactating females in cooperative systems nurse only their own offspring and as there are many more helpers present in the nest relative to the number of dependent offspring, lactating females have more time to feed, rest and replenish their energy. There was no evidence however that lactation length is reduced for communally caring females compared to other polytocous species, suggesting that the benefits gained from shared parental care are balanced by the increasing demands faced with caring for non-offspring. Offspring of communally breeding females also reach independence later than other polytocous species, suggesting that females may need to provide care for longer periods due to scramble competition between offspring.

Litter size positively influenced protein content of milk produced by females in both cooperative and communal systems. However there is an overall reduction in protein content in cooperative species, which potentially provides further potential evidence that mothers reduce costs associated with nursing in this system (see Konig, 2006; Landete-Castillejos *et al.*, 2005; Langer, 2008; Riek, 2011). Fat content of milk is not reduced in cooperative or communal species, but is negatively influenced by ecological traits which may have affected the diet of lactating females. Post natal offspring growth rates in cooperative species are not negatively affected compared to other polytocous species,

suggesting that both milk quality and food provisioned by helpers is sufficient to maintain growth of offspring born to cooperative females. Within cooperative systems, help normally consists of babysitting in or close to the nest site, guarding or protecting offspring and providing food such as invertebrates (e.g. Clutton-Brock *et al.*, 2003). There is however evidence that reproducing subordinate females may also provide milk for the offspring of dominant females. This is more likely to occur when the subordinate female is a sibling rather than a mother to the dominant individual, and in relatively small groups (Clutton-Brock *et al.*, 2003). This behaviour may be a form of appeasement by the subordinate female in 'payment' for reproduction, but as non-offspring nursing occurs between siblings, subordinates also gain indirect fitness benefits as a result. It would be interesting to further investigate the distribution of nursing performed by dominant and subordinate individuals in the cooperative nest, as it may provide further evidence in support of selection for reduced lactation length. As there is no difference in milk quality of communally breeding species compared to other polytocous species, it could be suggested that milk demand is not excessively increased in line with potential offspring demand, due to the presence of other lactating females. In addition, lactating females may be able to maintain milk quality due to the increased intervals between nursing, and increased time to forage (Hayes, 2000).

7.5.5 Influence of ecological factors on reproductive and life history traits

Body size dimorphism may be affected by species residing in particular ecological conditions or geographical locations in both birds (e.g. Arnold & Chen, 2009; Jetz & Rubenstein, 2011) and mammals (Isaac, 2005; Swihart *et al.*, 1988), as Bergmann's Rule predicts that species living in warmer climates are more likely to be smaller than species from colder regions (see Isaac, 2005). Ecological traits appeared to influence litter size at birth for both cooperative and communal species, as there was a negative relationship with average temperature. This finding complements previous studies that suggest cooperative care evolved in harsh climates, where access to resources could be unpredictable (Jetz & Rubenstein, 2011). Ecological traits were also important in predicting offspring development, as the reported age at which the offspring of cooperative and communal species reached sexual maturity was negatively influenced by average temperature.

Although data were collected on home range and average annual temperature and precipitation levels in this study, there was not sufficient data to include in the analysis

each model. It would therefore be interesting to extend the data set to increase and include more ecological factors when examining effects on life history and reproductive traits in both cooperative and communal species.

7.6 Conclusion

The potential for competition is increased in species with cooperative or communal care of young due to high reproductive skew or increased potential for offspring competition. Consequently selection may influence a number of reproductive and life history traits, which may vary between the two breeding systems. Despite greater potential for intra-sexual competition between cooperatively breeding females for reproductive opportunity, there was no evidence of reduced sexual size dimorphism compared to other polytocous species. However there was evidence of increased litter size and shorter inter-birth intervals in cooperatively breeding species, as well as a reduction in investment during the lactation period. Surprisingly, sexual size dimorphism was reduced for communally breeding species, despite the relatively lower potential for competition between females for reproductive opportunities compared to cooperative breeders. Offspring of communal breeders show some evidence of increased growth in-utero, as predicted under increased competition, and reach age of independence later than other species. Ecological conditions also influence life history traits in both cooperative and communal species, providing further evidence that cooperative breeders are more likely to be found in harsh climates. Expanding the data set to include more ecological factors and additional species data for lactation traits and oestrus cycle length may provide further insight. However, these results suggest that competition within cooperative and communally caring species has important consequences for the evolution of life history traits.

Chapter 8 General discussion

Female competition has received relatively little attention compared to male competition, due to the relative intensity for competition for breeding partners in the two sexes (Clutton-Brock, 2007; Cunningham & Birkhead, 1998; Stockley & Bro-Jørgensen, 2011; Trivers, 1972). But competition between females can also have important reproductive consequences. There are an increasing number of studies describing conditions where females compete to obtain resources necessary for reproduction, such as food or nest sites. Gaining access to these resources may lead to competition for high-quality mates that can also provide protection for females and their offspring. Intra-sexual competition for mates can also occur under conditions where sperm may be limited or as an anticipatory strategy to prevent future resource competition (see Stockley & Bro-Jørgensen, 2011 for a review). Evidence of competition exists even among communally breeding species, where relationships are thought to be relatively egalitarian due to low reproductive skew between group members. Females rearing young communally can show high levels of aggression around the time of parturition, particularly if birth is asynchronous between group members (Ebensperger, 1998; Ghiraldi *et al.*, 1993; Maestripieri, 1992), and there are a number of examples where female mammals inhibit the reproduction of other individuals (see Wasser & Barash, 1983). The principle aims of this thesis were to explore the extent of competition between females and to investigate the physiological and reproductive consequences of competition in a communally breeding species. I also used a comparative approach to examine if life history traits are influenced by competition in communally and cooperatively breeding species. In this chapter I will provide a brief summary of the main results presented in the preceding chapters and discuss them in the context of the broad themes introduced in Chapter 1. I also discuss some of the limitations of this thesis and possible directions in which this work could be expanded.

8.1 The importance of body mass in predicting competitive behaviour in female house mice

Female body mass is variable in house mice, which may be due to genetic, social and/or environmental factors. However variation between females is not just a consequence of laboratory housing; it is also found in wild populations. Within the stock population at MBE there is variation in weight between sexually mature individuals with similar social

and reproductive experience; relatively small reproductively inexperienced females can weigh approximately 12 g and larger females around 24 g, while reproductively experienced females can be in excess of 30 g. In a report by Berry (1970), average reported body mass for populations at various locations in the U.K. ranged from 16 to 26 g, which is also consistent with my own personal observations when trapping animals at a local safari park. In the laboratory, females have *ad. lib*. access to food and water, although it is possible that more competitive females could attempt to restrict food access for less competitive cage mates. In feral and commensal populations, access to food is reduced compared to laboratory conditions, but is not a limited resource due to access to stored foods and animal feed (Berry, 1981; Hurst, 1986; Hurst, 1987). It is therefore unlikely that diet would significantly influence variation in body mass in house mice. Health status can also affect body mass as wild populations are exposed to pathogens and parasites. The presence of parasites for example could influence growth rates during early development, consequently affecting competitive behaviour (for example Reed *et al.*, 2012).

A relationship between body mass and competitive ability has been demonstrated across a range of mammalian species for both males and females, as body mass is generally correlated with strength and ability to win contests (for examples see Chapter 1). In many species there is a positive association between age and body mass, and therefore age related hierarchies also correlate with body size. In house mice however, female age was not found to be correlated with aggressive behaviour at introduction, but there was a strong positive association with body mass (Chapter 3). Therefore under conditions where competition for reproductive resources could be increased (i.e. among communally breeding species), selection may favour increased body mass in females. The results of the comparative analysis conducted in Chapter 7 revealed this to be the case, as sexual size dimorphism was reduced for communally breeding species compared to other polytocous mammals. The influence of body mass on competitive behaviour in this thesis is therefore unlikely to be an isolated case.

During free-ranging interactions, females are likely to encounter a diverse range of individuals that are heavier or lighter, older or younger, related or unrelated to them. Signals of identity are sufficiently strong enough in house mice that individuals can detect if an encountered stranger is related to them, even through odour cues (Sherborne *et al.*, 2007; Thom & Hurst, 2004; Thom *et al.*, 2008b). Although urinary components could signal competitive ability (discussed below), body mass difference could also be assessed

between two novel mice when they approach to sniff each other on their first encounter. Body mass could therefore be a relatively quick and accurate way of measuring and comparing competitiveness between potential nest partners.

8.2 Signalling competitive ability in house mice

Aggressive behaviour is costly and should therefore only occur when establishing a new relationship with a social partner. Signals of competitive ability are likely to be less energetically demanding and also less risky than overt aggression, therefore selection may also favour competitive signalling in species where the potential for competition is high. In house mice, olfaction is the predominant method of communication. Male mice deposit urinary scent marks to advertise dominance status and territory ownership to other males, whereas females are thought to use odour cues to advertise fertility (Hurst, 1990b; Hurst, 1990c; Hurst, 1990d). Female house mice were found to deposit scent marks in response to male presence and, in agreement with studies of male signalling, there was evidence that more competitive females decreased the size of their scent marks following competitive interaction with another female (Chapter 4). However the frequency of scent marks deposited were reduced, which may be a consequence of energetic demand and/or cost of the components contained within the signal. A major component of house mice urine is protein, which was found to significantly increase for more and less competitive females following competitive interaction (Chapter 4). If scent marks contain clear signals of competitiveness then it may not pay to increase the frequency of marks over a relatively short period of time, due to the associated costs of production. Scent marking behaviour described in this thesis was assessed experimentally for older females over 12 months, but not for younger females. Older females had particularly high levels of urinary protein, even before they had experienced competitive interaction, whereas younger females produced protein at around two-thirds of the rate of older females. It would therefore be interesting to compare scent marking rates of females across the various age groups used in this thesis, to see if there are age related effects in scent mark production.

Strength (and characteristics) of urine signals in male mice are influenced by the presence of major urinary proteins (discussed below) and the volatile compounds released from the preputial gland (see Chapter 1). The function of the female clitoral gland is less well known, but evidence suggests that gland secretions could contain farnesenes and squalene which function to communicate identity and social status in males (Kannan & Archunan,

2001). The preputial gland of dominant males can be twice as large as those of subordinates, which may be the result of increased production of farnesenes and squalene (Novotny *et al.*, 1990). In this thesis, there was evidence to suggest that females previously housed with unrelated competitive social partners had enlarged glands compared to females previously housed with siblings from birth (Chapter 4), suggesting that females may use gland secretions in competitive environments. Further experiments could examine the gland secretions of house mice to identify volatile components and investigate if more competitive females increase investment following competitive housing.

Major urinary protein signalling was investigated for a range of female pairs to measure the degree of similarity in peak profiles. However, changes in the relative intensity of peaks were examined only for older females. Although little is known about MUP signalling in females, there is evidence that the MUP concentration varies throughout the oestrus cycle (Stopka *et al.*, 2007). A recent study also showed that MUP concentration can increase following territorial defence in both male and female house mice (Garratt *et al.*, 2012; Garratt *et al.*, 2011a). In the limited examination of MUP expression in this thesis, females appeared to alter the strength of relative intensity at particular peaks, which could change the characteristic of the signal, perhaps as a result of changes in social status. MUPs expressed by males at specific masses are important for attracting mates; for example darcin is expressed at mass 18,893 Da and has been shown to elicit female attraction and memory to the location of male scent (Roberts *et al.*, 2012; Roberts *et al.*, 2010). Although female specific peaks have not (yet) been identified, MUP signalling could potentially be used by females to signal characteristics other than reproductive status. Recently it was reported that a combination of MUP and the major histocompatibility complex (MHC) could be important for social partner choice (Holmes, 2012). Although no relationship was found between MUP sharing and competitive behaviour (Chapter 3), there was a positive relationship with reproductive output (Chapter 6). This is particularly interesting as female pairs in this experiment were all unrelated. If females perceive their social partner to be more similar to themselves over a relatively prolonged period (rather than at initial introduction), then it may pay to communally rear offspring and share the costs of parental care. The importance of MUP signalling for female house mice in competitive conditions is therefore an exciting and interesting area to investigate further.

8.3 Potential effects of social 'stress' on competitive signalling

The increased adrenal gland size observed in relatively competitive females (Chapter 4) suggests that the establishment of new social partnerships may have been stressful (see Chapter 1 for examples where this occurs). The adrenal glands are responsible for production of sex steroids such as testosterone and glucocorticoids such as corticosterone (Sapolsky, 2002). Increases in production of either of these hormones could have led to an increase in adrenal gland size (see Chapter 1). Although there are examples where high testosterone levels in females can result in more aggressive behaviour in other species, the relationship between testosterone and social status has predominantly been investigated during male competition (e.g. Clutton-Brock *et al.*, 2006; Dloniak *et al.*, 2006). Prenatal exposure to relatively high levels of testosterone has previously been shown to affect anogenital distance and delay sexual maturity in mice (Vom Saal, 1978). In this study, urinary testosterone had a strong positive influence on the amount of aggressive behaviour performed at introduction (Chapter 3), and urinary testosterone was observed to increase following competitive interaction (Chapter 4) highlighting the important influence of adrenal hormones on competitive behaviour. Measurements of other sex steroids produced in the adrenal gland, such as progesterone and oestrogen, may have revealed some parallels with competitive behaviour on first encountering another female, although there is limited and conflicting evidence of this elsewhere (e.g. Davis & Marler, 2003; Kapusta, 1998; Partecke & Schwabl, 2008). In this thesis, corticosterone output was not measured and therefore it is impossible to say whether adrenal gland size was affected by increased production of testosterone, or a combination of steroids. However, increases in circulating testosterone can have an impact on immunocompetence (Barnard *et al.*, 1996) and fertility (Franks, 1995; Packer *et al.*, 1995), and therefore females may have suffered associated costs of competition; although there was no apparent influence of testosterone on reproductive output of house mice in this thesis (Chapter 6; see below).

8.4 Influences of female competition on male mate preference

During mate choice experiments, males showed a mating preference for less competitive females although they did not discriminate between competitive partners on the basis of scent alone, even following female competitive experience (Chapter 5). This suggests that if females signal their competitiveness in their urine, it may not be used by males to choose a mate. Gland secretions may not have been present in the urine due to the collection method, and consequently signals produced in the clitoral gland are unlikely to be investigated by males. However, as both females were unrelated to the male he may have simply been attracted to both. When freely interacting with females, males approached less competitive females more frequently than more competitive females prior to mounting, and less competitive females were usually receptive (although this was not directly tested). When given limited access to females through a wire mesh barrier however, males were more likely to spend time with females that interacted with them more frequently, which both females were equally likely to do as females could not contact one another. The length of these trials however was relatively short and a clearer mate preference may have been observed over a 48 hour period (as observed in unpublished work by Holmes, 2012).

During mating trials, more competitive females sometimes interrupted mating attempts, although this did not appear to influence future male mounting behaviour. Less competitive females may be more receptive and less aggressive on approach and as a result are pursued first. When examining reproductive behaviour in a free-ranging environment it is however difficult to clearly distinguish between male preference, female receptiveness and the potential for female manipulation during mating; but in free-ranging environment, reproductive events are likely to be influenced by a combination of all three factors. Although behaviour was observed during mating trials there was no measure of ultrasonic vocalisations (USVs) between individuals within each group, which may have revealed more information on signalling between individuals. In a recent experiment using wild derived mice, Musolf *et al* (2010) found that males used courtship calls which females responded to, particularly if they were from an unfamiliar and unrelated male. It is possible that females respond differently to these calls, and perhaps respond in turn. There may even be differences in calls according to social rank. Very little work has been conducted on the use of USVs in wild house mice and therefore may reveal other signals or cues of social status that are used between individuals. The social status of the male may also

influence courtship calls and mating attempts. Bolder, more dominant males may be more willing to pursue a more competitive female, whereas more subordinate males may prefer less aggressive females.

8.5 Influences of female competition on reproductive output

Body size has a positive association with fecundity in female mammals (see Chapter 1), and in house mice there was a strong relationship between body mass and reproductive output during solitary breeding conditions. Reproductive output was however significantly reduced when competitive pairs of unrelated females communally reared their offspring compared to previous output in a solitary condition prior to competitive experience (Chapter 6). A higher frequency of more competitive females gave birth in the communal nest, however there was no difference in average litter size or pup survival rates between more and less competitive females. Heavier females tended to have increased reproductive output, but body mass asymmetry between female pairs did not influence differences in pup survival rate. When there was an age difference, pup survival rate for both females within a pair was higher than between females that were similar in age. However there was no relationship between competitive rank and reproductive output, suggesting that both females were negatively affected by competition. It would have been interesting to examine reproductive output in solitary conditions following competitive interaction to determine if females suffered a reduction in reproductive output as a result of the competition they experienced, or if the presence of a competing female was the main influential factor. However, comparative analyses conducted in this thesis (Chapter 7) found no evidence of increased litter size in communally breeding mammals compared to other polytocous species, despite the presence of other females to share the costs of parental care. This suggests that the potential for competition between females (and/or offspring) may have negative reproductive consequences, although ecological conditions can also affect reproductive success.

Reproductive opportunities were provided to experimental mice four days following competitive interaction and therefore females may still have been establishing their social hierarchy during this time, which may have increased competition for mates. Increases in body mass were detected for older competitive females between seven and 14 days following competition (Chapter 4) , which may be an indication of the time it takes females to adjust to a new social partner, particularly when they are similarly aged. In addition, a

single reproductive event may not have been sufficient to identify the long-term effects of competition on reproductive success. In long term studies of house mice in Switzerland, Konig and colleagues have examined reproductive success over six month periods as this may represent an average reproductive lifespan for house mice; only over this time period did females achieve increased reproductive success as a result of nesting communally compared to solitary breeding conditions (see Konig, 1994a; Konig, 1994b; Konig, 2006; Konig & Lindholm, 2012). However, females appeared to be motivated to nest together and pool their young, despite the opportunity to use either of the two nest boxes provided during the rearing period. This suggests that the benefits of communal rearing such as shared parental care, outweigh the potential costs (and risks) of nesting with a competitive social partner.

8.6 Competition between offspring

Offspring competition is likely to increase in conditions where the number of dependent young exceeds the number of nipples (Mock & Parker, 1997). The presence of multiple females in communal nests should increase the probability of access to milk and consequently reduce competition between offspring. However, females do not usually nurse simultaneously in the communal nest (Chapter 6), resulting in increased competition between littermates for access to nipples. Competition between females over the level of investment they provide can increase both parent-offspring conflict (Trivers, 1974) and competition between offspring. In Chapter 6 there was evidence for a reduction in offspring care by the most competitive female (when correcting for total number of pups present in the nest). The distribution of care between females may therefore vary according to competitive rank. Comparative analyses revealed no difference in milk protein or lipid content of communally nursing females compared to other polytocous species, however under experimental conditions Konig *et al* (1988) found that lipid concentration was lower for solitary nesting house mice with relatively large litters in the five to eight days following birth. If there were differences in lactation investment, milk quality could vary between more and less competitive females. This could be investigated by collecting milk from mothers using mechanical techniques similar to those used by Konig *et al* (1988). The distribution of milk could additionally be influenced during scramble competition between pups. Milk labelling experiments can be used to examine distribution of milk between litters by provisioning females with specific radio-isotope labelled food

(Thornburn & Bailey, 1983), although this is not a particularly cost effective method. Urine from offspring is analysed for the presence of isotopes to determine which female(s) they nursed from and the difference in distribution of isotope. If quality of milk is variable between females then offspring may compete for priority access to the female with the highest quality milk. Alternatively offspring may attempt to nurse primarily from their own mothers. However, there is an argument that offspring should attempt to nurse from all available females whenever they can as this will likely lead to increased growth, reinforcing competitive ability between littermates.

In terms of pup development in this thesis, the offspring of older females tend to gain more weight between birth and weaning (Chapter 6). In addition, offspring of the first litters born also gain more weight during this period, suggesting that older and heavier pups had a competitive advantage over lighter offspring. In most mammal species females are unable to selectively nurse offspring, and instead adopt a nursing position over the entire litter (Hudson & Trillmich, 2008). Offspring then scramble for access to teats, sometimes competing for access to more productive mammae, and remain attached until the nipple is temporarily depleted. In house mice, females rarely nurse simultaneously and instead take turns adopting nursing positions. Less competitive offspring therefore may not be able to nurse until another female enters the nest, and then they still may have to compete with other pups.

Measuring the total time females spend in contact with litters is a relatively non-invasive method and has been used in studies of African spiny mice (*Rhabdomys pumilio*) (Kinahan & Pillay, 2008), however it may not allow for a true depiction of maternal investment between females. Animal welfare is a majority priority in the design of behavioural experiments, as unduly stressing animals can result in both unnatural behaviour and in an increase in the stress levels of the mother. If female mice perceive a threat to their nest site (and therefore their offspring) they can perform infanticidal behaviour. As ultrasonic vocalisations are used by both mothers and offspring in house mice (Branchi *et al.*, 2004), recording USVs could be used as an additional method of measuring maternal care and mother-offspring interactions. Offspring can alert lactating females when they are hungry or if they leave the safety of the nest site and need retrieving. Mothers also emit USV in response to pups and therefore USVs are a simple way of recording communication between individuals in the nest. Offspring of more competitive females may vocalise more extensively to encourage the mother to adopt her nursing posture; these pups may also gain

advantages in scramble competition, particularly if they are larger or have inherited genetic components of competition from their mother.

Although not directly tested in this thesis, there was some evidence that females may have adopted maternal strategies to enhance the reproductive success of their offspring. Daughters of competitive mothers had larger average litter size over three consecutive litters compared to daughters of less competitive females. In addition, daughters that were born in competitive communal nests were more likely to have male biased litters, but the competitive rank of their mothers did not appear to influence litter size. There was no direct evidence to suggest that experimental females altered sex ratio of litters under competitive conditions compared to solitary, however infanticidal behaviour prevented examination of all the litters to determine gender of pups. Less competitive females showed no reduction in total litter mass at weaning from solitary to communal environments despite a reduction in litter size, which may suggest that less competitive females increase investment in fewer offspring which may improve their chances of survival or competitive ability. However, it is difficult to make any firm conclusions due to the significant reduction in reproductive output observed in the communal nest and using data from a single reproductive event. By extending reproductive success over an average lifespan, a more thorough investigation of maternal effects could be made.

8.7 Concluding remarks

The evidence presented in this thesis illustrates the significance of competition between female house mice and highlights the importance of body mass in competitive interaction. Clearly there is selective pressure on increased body mass for communally nesting species, which may enable females to compete for access to mates and other necessary resources. Whether this can be called sexual selection depends on how sexual selection is defined. Female competition for resources is important for female survival and maintenance, which consequently impacts on reproductive success. The distinction between natural and sexual selection is therefore blurred and is likely to be debated for some time (Clutton-Brock, 2009b; Stockley & Bro-Jørgensen, 2011).

Definitions aside, body mass provides female house mice with an advantage during competitive interaction, which may lead to accessing the best nest sites in a territory of the highest quality male. However, competitive interactions between nesting partners can lead

to serious negative consequences on reproductive output, even if males do prefer less competitive females during mating opportunities. This illustrates the importance of examining the whole structure of a social group and not just the extravagant behaviours or traits that are immediately obvious and visible. Subordinate behaviours were frequently observed throughout the experiments in this thesis and gave just as much information on the social dynamics of paired females as overt aggression could. Subordinate postures were quickly established in the presence of a more competitive individual, which may help to signal rank position, and reduce the risk of directed aggression from the more dominant female. Where contests were escalated, subordinate behaviour would only be observed following an attack or fight and this would be continue until the attacking female 'lost' the fleeing female or had spent a short amount of time resting in a higher position than the 'losing' individual. Reproductive output has remained the focus for many studies as overt aggression is not always observed. Overt aggression is costly for species with high reproductive costs and therefore signals of competitiveness, such as body posture or chemical communication are more likely to reveal differences between females, as illustrated throughout this study. The benefits of nesting with kin have been widely credited (e.g. Konig, 1994b), but females may not always have that choice, and even if they do there is evidence that they will compete with one another (Rusu, 2004). Mothers in the communal nest are more related to their own young than their sister's offspring, unlike eusocial insects, and therefore females may gain more through their own reproduction rather than helping a relative to rear offspring.

The potential for competition exists throughout life, during formation of social relationships with nest partners, when seeking mating opportunities, and also throughout gestation and lactation. Communal breeding increases cooperation between group members and allows individuals to share costs associated with parental care; but living within such a system can also result in increased competition between females. This thesis has attempted to progress knowledge of the existence of female competition in a communally breeding species, examining potential competitive traits and the physiological and reproductive consequences of competition, while the comparative approach illustrated the evolutionary implications of competition in terms of sexual size dimorphism and other life history and reproductive traits. Although there are various other aspects of competition to explore within communal breeding systems, this study highlights the importance of

examining the extent of female competition and its implications throughout breeding life using a novel combination of both behavioural and biochemical methods.

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Appendix

Comparative analysis data

The following table contains data used in the analyses for Chapter 7. The data were collected from a variety of sources which are referenced in the table and listed below:

1 Barton, R. A. & Capellini, I. 2011. Maternal investment, life histories, and the costs of brain growth in mammals. *Proceedings of the National Academy of Sciences*.

2 Ebensperger, L. A. & Hayes, L. D. 2008. On the dynamics of rodent social groups. *Behavioural Processes*, 79**,** 85-92.

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	Monogamous	Body mass dimorphism	Litter size (birth)	Offspring mass (birth)	Inter-birth interval	Gestation length	Oestrus cycle length	Lactation length	Milk fat content (%)	Milk protein content (%)	Offspring growth rate (per day)	Offspring growth rate in-utero	Age at independence	Age at maturity	Sources
Cooperative species															
Alopex_lagopus	1	0.81	7.80	58.50		52.00		63.00				1.13		270.00	4, 5, 8, 9
Atherurus_africanus	1	0.93	1.50	146.25		108.00		45.37				1.35			3, 4, 5, 8, 10
Atherurus_macrourus	1		1.50												4, 5, 8, 13
Callithrix_jacchus	1	1.02	2.31	27.74	159.80	142.89	21.33	60.20			1.15	0.19		456.00	1, 4, 5, 8, 13
Canis_aureus	1	0.87	3.74	211.80	365.00	61.24		61.30	10.50	10.00		3.46		371.20	5, 8, 9
Canis_latrans	1	0.85	5.72	200.01	365.00	61.74		43.71	10.70	9.90		3.24	255.00	372.90	5, 8, 9, 14
Canis_lupus	1	0.78	4.98	412.31	365.00	63.50		44.82	8.30	9.50		6.49	180.00	679.37	1, 5, 8, 9, 12
Canis_mesomelas	1	0.91	3.89	177.20	273.75	62.50		34.10				2.84	270.00	241.40	5, 8, 9
Canis_simensis	1	0.81	4.00		365.00	63.60		69.60					180.00	754.74	5, 8, 9
Castor_canadensis		1.11	3.60	415.20		112.00		46.50				3.71	729.99	663.00	3, 4, 5, 8, 10
Castor_fiber	1	1.28	2.95	550.30		106.70	12.42	60.18			60.65	5.16		1034.60	1, 4, 5, 8, 10
Cryptomys_damarensis	1	1.01	3.00	8.49				18.08							5, 8, 10
Cryptomys_hottentotus	1	0.79	2.48	8.24		84.99		32.07			0.52	0.10		472.60	4, 5, 8, 10
Cryptomys_mechowi	1		2.60	18.77	122.00	104.00		38.49				0.18			5, 8
Cryptomys_ochraceocinereus	1		3.00												4, 5, 8
Helogale_parvula	1		3.49		141.94	54.08		21.00						423.04	5, 8
Heterocephalus_glaber	1		9.00	1.88		63.17		33.33			0.27				4, 5, 8, 10
Leontopithecus_chrysomelas	1	0.86	2.04												5, 8, 13
Leontopithecus_rosalia	1	0.96	1.94	51.89	182.50	134.00	17.35	75.69	10.20	3.00	1.49	0.39		890.34	4, 5, 8, 13
Lycaon_pictus	1	1.02	8.10	297.45	355.87	71.18		27.28	9.50	9.30		4.18	390.00	817.15	5, 8, 9
Marmota_caudata	1		3.65			30.74		34.80					714.87	909.33	2, 5, 10
Marmota_marmota	1		4.00	29.75	912.50	35.22		49.18				0.84	1251.02	827.69	2, 4, 5

Meriones_unguiculatus	1		5.01	2.85	46.43	25.09	4.60	23.95			0.43	0.11		56.50	4, 5, 8, 10
Microtus_ochrogaster	1		3.87	2.94	26.00	21.50		21.00			0.58	0.14		36.19	5, 8, 14
Microtus_pinetorum	1		2.47	2.42	26.05	23.99		20.08			0.36	0.10		74.82	5, 8
Peromyscus_californicus	1		1.94	4.31	24.00	32.95	7.70	29.83			0.45	0.13		50.00	4, 5, 8
Peromyscus_polionotus	1	1.08	3.59	1.54	24.25	23.80		24.24			0.18	0.06		29.98	4, 5, 8, 10
Pseudomys_albocinereus	0	0.74	3.89		38.50	29.75		29.58					29.58	77.34	3, 5, 10
Rhabdomys_pumilio	1	0.98	5.28	2.50	29.07	25.44	11.00	14.00			0.42	0.10		61.87	4, 5, 8, 10
Saguinus_bicolor	1	1.00	2.00		195.00	158.16									5, 8, 13
Saguinus_midas	1	0.74	2.02	39.78	206.83	138.24	16.00	69.60				0.29		841.82	4, 5, 8, 13
Saguinus_mystax	1	1.06	1.93	46.65	168.50	148.30						0.31		556.85	4, 5, 8, 13
Saguinus_oedipus	1	0.97	1.90	41.00	233.06	166.17	21.95	49.85			1.28	0.25		680.38	4, 5, 8, 13
Suricata_suricatta	1		3.86	30.50	365.00	77.00		55.68			3.50	0.40		377.37	5, 8, 12
Communal species															
Acomys_cahirinus	0	1.31	2.43	5.49	34.00	38.54		14.00			0.49	0.14		51.90	3, 4, 5, 8, 10
Antrozous_pallidus	0		1.78	3.09		59.01					0.17	0.05	61.10		5, 7, 8
Ateles_geoffroyi	0	0.94	1.01	425.85	1048.15	226.37					3.74	1.88	816.35	2104.57	5, 7
Baiomys_taylori	0		2.52	1.15	23.00	21.99		20.38				0.05		68.35	3, 5
Callimico_goeldii	0	0.94	1.05	50.50	183.00	154.00	23.00	66.50			1.05	0.33		413.80	5, 8, 13
Cavia_porcellus	0		3.45	79.91	96.30	66.99	16.00	15.75			8.41	1.19	16.68	68.59	1, 3, 5, 8, 12
Cebus_olivaceus	0	0.77	1.01		792.05			725.86						2525.48	4, 5, 8, 13
Cervus_elaphus	0		1.09	8255.82		235.61		120.00	7.60	6.40	297.68	35.04	104.64	659.91	1, 5, 7, 8, 12
Crocuta_crocuta	0		1.91	1460.00	441.04			371.37			35.11		913.00	789.54	5, 8
Ctenodactylus_gundi	0		2.00	29.89	69.70	65.96						0.45		169.03	2, 5
Cynomys_gunnisoni	0	0.76	4.48	4.14		29.64		36.65			0.02	0.14		395.29	4, 5, 13
Cynomys_ludovicianus	0	0.95	4.43	15.24		33.46		45.57			2.38	0.46	524.89	696.90	3, 4, 5, 8, 14
Dolichotis_patagonum	1		1.75	578.96	99.61	97.97		76.28				5.91		216.03	3, 4, 5, 7
Galea_musteloides	0		2.60	37.39	53.10	53.90		31.13			2.45	0.69		55.92	3, 5
Giraffa_camelopardalis	0		1.29	55244.90	608.33	455.25			4.80	4.00		121.35	212.91	1633.59	5, 7
Glaucomys_volans	0	1.06	3.13	3.61	116.75	39.49		57.23			0.74	0.09		335.63	5, 7, 10

Gorilla_gorilla	0	0.05	1.05	2095.89	1430.80	257.00	27.01	920.00	1.50	1.20	18.34	8.16	5248.98	3353.12	4, 5, 11, 13
Hydrochaeris_hydrochaeris	0	0.98	3.49	1550.00	109.75	150.70		112.50				10.29		566.40	3, 4, 5, 8, 10
Hylochoerus_meinertzhageni	0		4.00		365.00	151.00		62.82					198130.47	521.59	4, 5, 8
Lagostomus_maximus	0	0.58	1.93	196.00		155.70	43.00	55.70			6.24	1.26	623.65	287.80	2, 4, 5, 10
Lagurus_lagurus	0		4.00	1.25	27.50	20.24		20.84			0.22	0.06		41.82	3, 5
Lemur_catta	0	1.00	1.18	75.80	468.41	134.74			2.30	1.90		0.56	126.51	831.62	5, 7
Macaca_mulatta	0	0.70	1.01	471.47	385.50	166.07			6.20	2.10	2.77	2.84	304.16	1101.07	5, 7
Macaca_sylvanus	0	0.69	1.02	449.85	431.71	164.84					8.89	2.73	210.25	1542.25	5, 7
Marmota_caligata	0		4.67			30.00		24.36					714.87	827.69	2, 4, 5
Marmota_flaviventris	0	0.72	4.66	33.79	456.25	30.39		25.00			16.16	1.11	357.43	827.69	2, 4, 5
Meles_meles	0		3.11	90.24	365.00	48.60		91.30				1.86	210.00	420.91	5, 8
Microcavia_australis	0		2.48	30.39	54.60	54.91	15.00	20.86				0.55		89.57	3, 4, 5, 8
Microcebus_murinus	0	1.07	2.00	4.79	365.00	60.34	47.20	40.45				0.08		355.53	4, 5, 8, 13
Microtus_arvalis		0.86	4.99	1.93	25.15	21.00	4.00	17.18			0.42	0.09		28.19	3, 4, 5, 10
Microtus_californicus	1	0.63	4.41	3.28		21.18		17.36			0.90	0.15		23.81	4, 5, 7, 10
Microtus_pennsylvanicus	0	0.90	5.16	2.40	21.19	21.24		12.92			0.83	0.11		31.74	4, 5, 7, 13, 14
Microtus_townsendii	0	0.76	5.06			23.20	8.00	15.44						51.29	4, 5, 8
Mungos_mungo	0		2.68	24.20	365.00	60.91		20.88				0.40		349.33	5, 8
Mus_musculus	0	1.07	5.54	1.06		19.60		21.50			0.42	0.05		64.71	1, 2, 3, 4, 5, 10, 12
Myocastor_coypus	0	0.95	5.34	205.70	131.50	131.86	17.00	52.80	27.90	13.70	29.25	1.56		187.09	2, 4, 5
Nasua_narica	0		4.00	140.00	365.00	76.82		132.63				1.82	365.00	1009.04	5, 8
Neotoma_cinerea	0	0.77	3.45	14.40	30.00	29.69						0.49	26.35	525.00	2, 5
Nycticeius_humeralis	0		1.94	1.73				28.30			0.10				4, 5, 8
Oryctolagus_cuniculus	0		5.24	39.11	29.00	30.45					6.54	1.28	26.30	185.61	1, 5, 7, 12
Ovibos_moschatus	0		1.01	10444.11	547.50	256.26					204.43	40.76	254.48	1262.74	5, 7, 12
Pan_troglodytes	0	0.81	1.05	1745.02	1825.00	231.49	26.50	1260.81	3.70	1.20	5.36	7.54		3897.96	4, 5, 7, 11, 13
Panthera_leo	0		2.75	1291.71	730.00	108.74		197.86	17.50	9.30		11.88	1080.00	987.77	1, 5, 6, 8
Parahyaena_brunnea	0			653.23	237.25	90.5		118.93					928.08		5, 8

Pecari_tajacu	0		1.56	618.53	155.00	144.88		47.25			87.94	4.27		315.68	5, 8
Peromyscus_leucopus	0	0.96	4.27	1.80	26.60	23.17	6.00	21.49			0.33	0.08	39.20	45.15	4, 5, 8, 10
Peromyscus_maniculatus	0	1.02	4.76	1.73	27.14	26.68	4.90	22.49			0.31	0.06		48.05	1, 2, 4, 5, 10
Phacochoerus_aethiopicus	0		3.20	691.95	365.00	165.40	42.00	106.45				4.18		63.12	4, 5, 8
Rattus_norvegicus	0	0.83	8.99	5.80		21.74		25.37			1.86	0.27		55.23	1, 3, 5, 10, 12
Rhombomys_opimus	0		4.67	4.75		27.50		22.00				0.17		105.00	2, 4, 5
Spermophilus_beecheyi	0	0.77	6.71	9.29		28.37		52.60			1.08	0.33	42.20	365.00	4, 5, 11, 14
Spermophilus_columbianus	0	0.70	3.54	9.58		24.09	14.50	32.19			3.08	0.40	28.50	625.91	3, 4, 5, 14
Spermophilus_parryii	0	0.91	6.50	11.00	456.25	25.16		28.00				0.44		420.91	4, 5, 8
Syncerus_caffer	0		1.08	39843.11	638.75	337.49					350.80	118.06	319.37	1683.65	5, 7
Tadarida_brasiliensis	0		1.11	3.19		89.99					0.15	0.04	54.24	400.26	5, 7
Varecia_variegata	0	0.99	2.61	92.53	365.00	101.20	34.30	90.00	3.20	4.20		0.91		600.00	1, 4, 5, 8, 13
Vulpes_vulpes	1	0.87	4.59	100.94	365.00	52.50		50.71	5.80	6.70	27.34	1.92	225.00	321.07	1, 5, 7, 10, 11
Xerus_inauris		1.02	2.06	20.00		47.29		51.85				0.42		347.80	2, 5, 10
Other polytocous species															
Abrocoma_cinerea	0		2.19	13.26		109.49						0.12			5, 8
Acinonyx_jubatus	0		3.28	441.66	547.50	92.24		125.00	9.50	9.40		4.79	91.42	741.97	5, 8
Acomys_cilicicus	0	0.84	2.62	5.44	34.00	38.97	11.05	18.60			0.52	0.14		54.31	4
Acomys_russatus	0	1.10	2.50												5, 10
Acomys_spinosissimus	0	0.98	2.71												5, 10
Acomys_wilsoni	0	1.18	2.29	3.49											5, 10
Acrobates_pygmaeus	0		2.80	0.01	183.00						0.04		97.50	272.11	5, 8
Aethomys_chrysophilus	1	0.89	3.09	4.43		26.17					1.07	0.17	24.88	96.05	5, 8
Aethomys_hindei	0		2.05			24.66								59.11	5, 8
Aethomys_kaiseri	0		3.00	6.09		27.00					1.29	0.23	26.00		5, 8
Ailuropoda_melanoleuca	0		1.62	104.39	638.75	134.99					122.34	0.77	178.98	2413.02	5, 8
Ailurus_fulgens	0		1.70	104.04	365.00	131.50		136.87				0.79	136.87	604.05	
Akodon_azarae	0		4.59			23.00							14.44	61.87	5, 8
Akodon_molinae	0		3.77	2.94	30.00	23.00						0.13	26.00	28.07	5, 8

Alces_alces	0		1.25	12999.99	365.00	235.00	110.00	10.00	8.40	666.36	55.32	98.85	668.20	5, 8, 12
Allactaga_bullata	0		2.52											5, 8
Allactaga_elater	0	0.81	4.47									32.31	105.22	5, 8
Allactaga_euphratica	0		4.99											5, 8
Allactaga_major	0		3.49									45.36	377.57	5, 8
Allactaga_sibirica	0		3.44											5, 8
Alouatta_palliata	0	0.74	1.02	318.29	684.37	185.42		1.80	2.30	1.48	1.72	495.60	1578.42	5, 8
Alticola_argentatus	0		4.57											5, 8
Alticola_strelzowi	1		6.90	3.00		20.85					0.14	17.00		5, 8
Ammospermophilus_harrisii	0		6.32	3.59		29.19					0.12	48.63	198.57	5, 8
Ammospermophilus_leucurus	0		8.00	2.90		29.38				0.50	0.10	64.51	377.37	5, 8
Ammospermophilus_nelsoni	0		8.90	4.87		26.00				1.21	0.19	29.83	377.57	5, 8
Antechinus_stuartii	0		6.89	0.01	365.00	28.15				0.18	0.00	89.74	329.99	5, 8
Antilocapra_americana	0		1.93	3454.70	365.00	247.99	105.00	13.00	6.90	59.12	13.93	87.54	534.68	5, 8, 14
Antilope_cervicapra	0		1.22	3386.14	212.56	166.59	59.50	1.30	6.90		20.33	59.50	700.04	5, 8
Aotus_trivirgatus	1	0.91	1.06	96.49	271.00	133.47				2.09	0.72	76.21	736.60	5, 8
Aotus_vociferans	1	0.99	1.02											5, 8
Aplodontia_rufa	0	0.78	2.46	23.12		30.00				5.55	0.77	58.40	827.69	5, 8
Apodemus_agrarius	0	0.97	5.64	1.80	19.50	19.89					0.09		76.04	5, 8
Apodemus_flavicollis	0	0.88	4.94	2.30	38.75	24.50					0.09		43.27	5, 8
Apodemus_sylvaticus	0	1.13	5.16	1.50	41.28	23.68					0.06	19.36	57.93	5, 8
Arborimus_longicaudus	0		2.91	2.49		28.11				0.61	0.09	30.49		5, 8
Arctocebus_calabarensis	0	0.98	1.01	31.82	144.47	133.74				1.17	0.24	109.26	298.91	5, 8
Arctocephalus_tropicalis	0		1.40	4347.41	365.00	241.69	304.16			34.59	17.99		1538.68	5
Arvicanthis_niloticus	0	0.87	4.86	4.25	26.50	21.99				0.86	0.19	21.00	51.89	5, 8
Arvicola_sapidus	1		3.21											5, 8
Ateles_belzebuth	0	0.95	1.02			138.20								5, 8
Ateles_fusciceps	0	1.03	1.01	499.99	851.66	224.70					2.23	482.70	1799.68	5, 8
Atilax_paludinosus	0		2.00	100.00	182.50	77.27				12.54	1.29	35.89	234.83	5, 8

Avahi_laniger	1	1.28	1.01		365.00	136.15							149.15		5, 8
Babyrousa_babyrussa	0		1.73	715.00		156.50	30.00	212.90				4.57		362.50	5
Baiomys_musculus	0		2.62	1.66											5, 8
Balaenoptera_borealis	0		1.02	649999.99	730.00	334.58						1942.73	203.46	3274.59	5, 8
Balaenoptera_physalus	0		1.01	1899999.9	730.00	338.36						5615.32	196.58	2666.41	5, 8
Bandicota_bengalensis	0		7.29	4.85	61.90	18.89					0.60	0.26	30.03	63.52	5, 8
Bandicota_indica	0	0.65	6.12	9.77	71.25	20.26					1.14	0.48	27.30	145.81	5, 8
Bathyergus_janetta	0		3.49	15.39									27.79		5, 8
Bathyergus_suillus	0	0.79	2.89	33.99		68.41						0.50	20.84		5, 8
Blarina_brevicauda	0		5.39	0.89		20.58						0.04	21.91	71.19	5, 8
Callicebus_modestus	1		1.02												5, 8
Callicebus_moloch	1	0.94	1.01	74.40	365.00	164.00						0.45	58.85	1262.74	5, 8
Callicebus_oenanthe	1		1.02												5, 8
Callicebus_olallae	1		1.02												5, 8
Callicebus_personatus	1	1.09	1.01												5, 8
Callicebus_torquatus	1	0.95	1.01										121.66	1683.65	5, 8
Callithrix_argentata	1	1.09	1.84	35.80	219.60									701.50	5, 8, 13
Callithrix_flaviceps	1		2.00			140.00							270.00		4, 5, 8, 13
Calomys_laucha	0		5.30			25.00									5, 8
Calomys_musculinus	0	0.78	6.27			20.97								76.03	5, 8
Caluromys_philander	0		4.18	0.20	166.50	23.99					0.19	0.01	119.65	314.52	5, 8
Cannomys_badius	0		2.00			41.49							50.10		5, 8
Capra_ibex	0		1.11	2779.07	547.50	167.50					115.48	16.59	111.75	838.54	5, 8, 12
Capreolus_capreolus	0		1.79	1208.55	365.00	196.00						6.17	79.75	400.97	5, 8
Caprolagus_hispidus	1		3.46			40.00									5, 8
Capromys_pilorides	0		2.00	199.49		125.00						1.60	41.99	314.64	5, 8
Catagonus_wagneri	0		2.47	600.20		152.08		60.37				3.95		821.58	4, 5
Cebus_albifrons	0	0.72	1.01	231.90	547.50	158.30	17.75	270.00			2.54	1.46		1501.70	4, 5, 8, 13
Cebus_apella	0	0.69	1.05	231.38	657.00	154.99	18.00	263.10	5.20	2.40	3.35			1760.81	4, 5, 13

Cebus_capucinus	0	0.69	1.01	238.36	790.80	161.06	514.07	2.16			2134.73	4, 5, 13
Cercopithecus_campbelli	0	0.60	1.02			180.80				362.93		5, 8
Cercopithecus_neglectus	1	0.56	1.02	259.95	584.00	172.07		3.14	1.51	417.62	2076.39	5, 8
Cercopithecus_preussi	0		1.02									5, 8
Cercopithecus_solatus	0	0.57	1.01		547.50							5, 8
Cerdocyon_thous	1	0.63	3.09	140.00	243.33	57.18	72.33		2.45	195.00	279.15	4, 5, 8, 9
Chaetodipus_baileyi	0		3.68									5, 8
Chaetodipus_fallax	0		3.45			25.00					168.83	5, 8
Chaetodipus_hispidus	0		5.34							30.18		5, 8
Chaetodipus_nelsoni	0		3.46			30.63						5, 8
Chaetodipus_penicillatus	0		3.40			26.00						5, 8
Cheirogaleus_major	1	0.83	2.26	18.08		70.00			0.26	47.14	420.91	5, 8
Cheirogaleus_medius	1	0.96	2.04	14.65	365.00	61.79			0.24	60.65	413.84	5, 8
Chelemys_macronyx	0	0.97	4.37									5, 8
Chionomys_nivalis	0	1.02	3.46	3.70	33.00	20.67	21.00				365.00	4, 5, 10
Civettictis_civetta	0		2.31	317.30	212.14	68.40		3.62	4.64	82.91	286.24	5, 8
Clethrionomys_glareolus			4.31	1.83	21.75	19.74		0.39		19.73	40.91	4
Condylura_cristata	0		5.39	1.50		40.00			0.04	20.84	304.16	5, 8
Crocidura_russula	0		4.04	0.80	28.50	29.00		0.21		23.82	66.88	5, 8
Crossarchus_alexandri	1		4.00									5, 8
Crossarchus_obscurus	1		4.29	9.78	121.66		29.23				278.42	5, 8
Cryptoprocta_ferox	0		2.98	99.96		92.54		37.85	1.08	136.10	1262.74	5, 8
Ctenodactylus_vali	0		2.00	17.90	64.00	59.13			0.30		340.64	5, 8
Ctenomys_peruanus	0		3.00	34.70		120.83			0.29		365.00	4, 5
Ctenomys_talarum	0	0.75	4.34	8.00		102.67	34.80		0.08		213.20	4
Cuon_alpinus	1	0.84	4.30	275.00	365.00	61.50	51.33		4.47	210.00	374.18	4, 5, 8, 9
Cynictis_penicillata	1		2.15		365.00	56.83	41.76			365.00	350.57	5, 8
Cynomys_leucurus	0	0.73	5.41			30.39	31.76				413.84	4, 5
Cynopterus_sphinx	0		1.50	11.00		120.00		0.43	0.09	32.49	312.99	5, 8

Dactylopsila_trivirgata	0		1.54		365.00							5, 8
Dasyprocta_leporina	0	1.12	1.40			106.39			140.00			5, 8
Dasyprocta_punctata	1		1.25		127.00	107.65			140.00			5, 8
Dasypus_novemcinctus	0		3.96	96.48		134.00		0.72	136.87	511.16		5, 8
Daubentonia_madagascariensis	0	0.95	1.01	121.79	760.41	166.48	7.15	0.73	197.70	834.72		5, 8
Dicrostonyx_groenlandicus	0		3.78	4.42	24.50	20.84	1.06	0.21	17.42	38.61		5, 8
Didelphis_aurita	0		6.11						99.71	192.74		5, 8
Didelphis_virginiana	0		8.62	0.15	136.87	12.69	1.14	0.01	109.62	225.55		5, 8
Dinomys_branickii	1		1.97	769.43		252.74		3.04				5, 8
Dipodomys_agilis	0		2.60							210.00		5, 8
Dipodomys_californicus	0		2.60									5, 8
Dipodomys_compactus	0		1.94									5, 8
Dipodomys_deserti	0		3.36	4.08		30.50		0.13	24.17	50.88		5, 8
Dipodomys_elator	0		2.91									5, 8
Dipodomys_heermanni	0		3.11	3.70		30.99	1.26	0.12	25.90	53.13		5, 8
Dipodomys_ingens	0	0.97	4.77									5, 8, 14
Dipodomys_merriami	0	0.86	2.39	3.29	54.00	30.77	0.67	0.11	20.42	67.11		5, 8
Dipodomys_microps	0		2.37	4.00		30.99	0.81	0.13	21.00	147.92		5, 8
Dipodomys_nelsoni	0		1.94									5, 8
Dipodomys_nitratoides	0		2.14	2.96		32.89	0.76	0.09	22.37	95.24		5, 8
Dipodomys_ordii	0	0.92	2.95	4.99	142.95	29.69		0.17		86.77		5, 8
Dipodomys_panamintinus	0		3.78	4.50		29.44		0.15	27.89			5, 8
Dipodomys_phillipsii	0		2.59									5, 8
Dipodomys_spectabilis	0		2.67	7.74	42.00	23.50		0.33	23.47	310.33		5, 8
Dipodomys_stephensi	0		2.68	4.40			1.11		19.86			5, 8
Dipodomys_venustus	0		1.73									5, 8
Dipus_sagitta	0		3.49			27.50						5, 8
Dolichotis_salinicola	0		1.94	199.00		77.50		2.57				5, 8
Dryomys_nitedula	0		3.24	1.57		24.50		0.06	31.32			5, 8

Echinops_telfairi	0		5.49	7.49		62.54					0.12	31.18	278.42	5, 8
Eira_barbara	0		2.14	83.00		66.74	94.46			16.65	1.24		766.15	5
Elephantulus_brachyrhynchus	1		1.54		75.00									5, 8
Elephantulus_edwardii	1		1.80	11.90								27.28		5, 8
Elephantulus_fuscus	1		1.39											5, 8
Elephantulus_intufi	1		1.55	10.00	67.00	51.00					0.20	36.49	251.19	5, 8
Elephantulus_myurus	1		1.84	8.10	49.00	50.66					0.16	22.82	39.82	5, 8
Elephantulus_rufescens	1		1.41	10.29	92.60	57.00					0.18	24.85	61.87	5, 8
Elephantulus_rupestris	1		1.84			56.00								5, 8
Elephas_maximus	0		1.41	97000.00	1186.25	634.49	810.00				152.88	218.26	4014.43	1, 5, 8
Eliomys_quercinus	0	0.80	4.99			22.97						34.80	566.36	5, 8
Euphractus_sexcinctus	0		1.73	104.98		64.66					1.62	28.00	283.18	5, 8
Felis_chaus	0		2.94	135.50	130.01	62.88				21.75	2.15	95.49	383.18	5, 8
Felis_silvestris	0		3.59	106.40	148.25	65.49				6.33	1.62	76.01	350.76	1, 5, 8
Galago_senegalensis	0	0.83	1.50	11.50	219.00	126.98		4.60	5.50	1.18	0.09	93.93	330.37	5, 8
Galemys_pyrenaicus	0		3.54			30.00							371.23	5, 8
Gazella_dorcas	0		1.22	1573.21	337.85	172.00	82.50	8.80	8.80	29.43	9.15	61.35	620.76	5, 8, 12
Genetta_genetta	0		2.29	77.75	182.50	74.18					1.05	71.80	1262.74	5, 8
Geocapromys_ingrahami	0		1.04	76.68	325.00	109.21				29.68	0.70	5.84		5, 8
Geomys_arenarius	0		4.71											5
Glirulus_japonicus	0		4.00			31.40						38.00	145.02	5, 8
Grammomys_dolichurus	1	1.15	3.11	4.19	38.00	23.99					0.17	23.87	83.05	5, 8
Graphiurus_murinus	1	0.89	3.00	3.49		23.99					0.15			5, 8
Graphiurus_ocularis	0		4.99		56.00									5, 8
Gulo_gulo	0		2.84	86.02	821.25	161.73	83.64				0.53	21.00	756.60	5
Gymnobelideus_leadbeateri	0		1.54		183.00	18.43						119.32	481.03	5, 8
Hapalemur_griseus	0	0.90	1.50	45.20	334.58	141.24		2.70	1.90		0.32	136.29	1003.17	5, 8
Helarctos_malayanus	0		1.10	324.99		98.34					3.30	90.36		5, 8
Heliophobius_argenteocinereus	0	0.85	2.46	7.00		87.00					0.08			5, 8

Hemicentetes_semispinosus	0		5.32	7.39		53.07					0.14	21.50	48.57	5, 8
Hemiechinus_auritus	0		3.74	9.50		37.18					0.26	39.70		5, 8
Heterohyrax_brucei	0		1.89	225.00	365.00	228.12					0.99	129.04	510.44	5, 8
Heteromys_anomalus	0		2.83	3.29										5, 8
Heteromys_desmarestianus	0		3.06	3.00		27.86					0.11	29.77	237.85	5, 8
Heteromys_gaumeri	0		2.95											5, 8
Hyaena_hyaena	0		2.44	653.23	237.25	90.50	118.93				7.22		928.08	5, 8
Hydropotes_inermis	0		3.00	1016.19		175.49					5.79		292.63	5, 8
Hylobates_agilis	1	0.99	1.02											5, 8
Hylobates_klossii	1	1.04	1.01			207.59								5, 8
Hylobates_lar	1	0.91	1.01	398.86	983.67	212.91		2.40	1.30	0.86	1.87	725.86	3852.57	5, 8
Hypogeomys_antimena	1	0.98	1.26										729.99	5, 8
Hypsignathus_monstrosus	0		1.40	39.30		125.14					0.31		227.99	5, 8
Hystrix_africaeaustralis	1	1.12	1.51	305.59	375.00	93.49					3.27	70.34	438.45	5, 8
Hystrix_cristata	1		1.73			79.19								5, 8
Hystrix_indica	1	0.91	1.73		109.00	112.00								5, 8
Ictonyx_striatus	0		2.30	13.69	365.00	36.76	59.09				0.37		447.70	4
Indri_indri	1	1.17	1.01		912.50	136.50						331.34	1605.69	5, 8
Jaculus_blanfordi	0		3.88											5, 8
Jaculus_jaculus	0	0.93	3.24	2.00	91.25	33.54					0.06	66.86	240.52	5, 8
Jaculus_orientalis	0	1.03	2.41			40.00								5, 8
Kerodon_rupestris	0		1.52	82.44	86.15	75.89					1.09	34.90	92.97	5, 8
Lagothrix_lagotricha	0	0.86	1.01	437.09	638.75	223.99					1.95	312.66	1729.33	5, 8
Lasionycteris_noctivagans	0		1.77	1.92		53.55				0.23	0.04	35.99	154.68	5, 8
Lasiurus_borealis	0		3.12	4.29		84.21				0.04	0.05	38.00		5, 8
Lemniscomys_barbarus	1		4.61											5, 8
Lepus_europaeus	0		2.14	123.00		41.99					2.93		210.95	5, 8, 12
Lepus_saxatilis	0		1.52	98.00		38.49					2.55			5, 8
Liomys_adspersus	0		3.20										202.08	5, 8

Liomys_pictus	0		3.67	2.50		25.16					0.77	0.10	25.90	111.11	5, 8
Liomys_salvini	0		3.69	1.90		27.68						0.07	24.81		5, 8
Lophuromys_flavopunctatus	0		2.30	6.50		30.70					0.21	0.21	42.72	61.02	5, 8
Lophuromys_nudicaudus	0		1.55												5, 8
Lophuromys_sikapusi	0	0.78	3.00	7.80							0.43		13.67		5, 8
Lophuromys_woosnami	0		1.97	9.50		32.00						0.30			5, 8
Loris_tardigradus	0	1.02	1.44	11.00	182.50	165.99			7.90	4.00	0.76	0.07	167.49	350.76	5, 8
Lutrogale_perspicillata	1		3.24			63.31		72.53						943.94	5, 8
Lynx_canadensis	0		2.73	204.00	365.00	62.50						3.26	111.36	672.90	5, 8
Lynx_lynx	0		2.30	289.00	365.00	66.99						4.31	81.85	739.52	5, 8
Lynx_rufus	0		2.76	300.00	365.00	59.99						5.00	59.99	668.22	5, 8
Macaca_fuscata	0	7.30	1.02	501.57	486.66	172.99			4.20	1.60	6.60	2.90	265.04	1460.77	1, 5, 8
Macaca_radiata	0	0.58	1.01	367.52	365.00	161.56					4.91	2.27	332.25	1785.78	5, 8
Macroscelides_proboscideus	1		1.93			58.49							4.99		5, 8
Marmosa_robinsoni	0		9.50	0.08	134.00	13.83					0.16	0.01	64.63	253.82	5, 8
Marmota_baibacina	1		5.99			40.00									4, 5
Marmota_camtschatica	1		4.99	32.99											4, 5
Marmota_menzbieri	1		2.73											943.94	5, 8
Marmota_monax		0.99	4.10	27.19		31.50		43.83			5.04	0.86	357.43	413.84	4, 5
Marmota_olympus		0.74	4.00					34.73					714.87	1141.75	5, 8
Martes_americana	0		2.60	31.30	365.00	27.63		45.73			7.77	1.13		456.25	5, 8
Martes_martes	0		3.49	30.00	365.00	30.63		54.19			12.00	0.98	157.50	508.47	4
Martes_pennanti	0		3.02	28.97	365.00	31.28		76.76				0.93	150.00	413.84	4
Massoutiera_mzabi	0		2.04	20.50		55.72						0.37		374.16	5, 8
Mellivora_capensis	0		2.35	210.00	182.50	181.46		23.86				1.16			4
Mephitis_mephitis	0		5.70	33.19	365.00	63.30		55.15	8.00	7.00		0.52	84.00	55.18	5, 6, 12
Meriones_crassus	0	0.90	4.21	3.35	67.00	23.50		30.24				0.14		68.97	4, 5
Meriones_libycus	0		4.54	5.25		25.43	5.00	30.18			1.32	0.21	30.00	92.80	4, 5, 10
Meriones_persicus	0		5.83	4.99		28.00		19.00				0.18			5

Meriones_tamariscinus	0		4.04	2.97	27.00			17.44			0.70				5
Mesocricetus_auratus	0		8.79	2.35		15.49		22.50	4.90	9.40	0.92	0.15	18.65	50.97	5, 8
Metachirus_nudicaudatus	0		3.87		365.00										5, 8
Microcavia_niata	0		3.16		55.00	54.00		20.88							5
Microcebus_rufus	0		2.52	6.50	73.00	59.99		40.00				0.11			4, 5, 13
Microdipodops_megacephalus	0	1.05	3.94												5, 8
Microdipodops_pallidus	0		3.89	1.00											5, 8
Microgale_talazaci	1		2.23	3.64		60.74						0.06	29.38	394.57	5, 8
Microtus_longicaudus	0		4.73											365.00	4, 5, 10
Microtus_montanus	1	0.71	5.54	3.89	22.25	21.13		15.00			0.36	0.18		24.91	3, 4, 5, 8, 10
Microtus_oregoni	0	1.18	3.33	1.69	38.75	23.80		13.91				0.07		34.23	4, 5, 10
Microtus_richardsoni	1	0.87	5.93	5.48		22.29						0.25			4, 5, 8
Microtus_xanthognathus	0	0.75	8.10	3.49				30.41							4, 5, 8, 10
Monodelphis_domestica	0		7.29	0.09	52.50	14.55					0.42	0.01	49.49	162.11	5, 8
Moschus_chrysogaster	0		1.20	659.54		188.00						3.51	47.14	587.94	5, 8
Mus_minutoides	1	1.06	4.18	1.01	31.20	20.71	4.00	20.90			0.11	0.05		57.35	4, 5, 8, 10
Muscardinus_avellanarius	0		4.30	0.80	50.00	24.97						0.03	37.61	346.11	5, 8
Mustela_frenata	0		6.50	3.09	365.00	24.50		34.23			0.75	0.13	84.00	200.69	5
Mustela_lutreola	0		4.50	7.40	365.00	43.74		58.71				0.17		351.71	5
Myoprocta_acouchy	0		1.97	75.23		98.43					10.49	0.76	26.19	218.76	5, 8
Myrmecobius_fasciatus	0		3.68		365.00	14.00							269.23		5, 8
Mystromys_albicaudatus	0	0.82	3.00	6.50	36.00	37.00					1.05	0.18	32.00	124.72	5, 8
Napaeozapus_insignis	0	1.07	4.50	0.93		22.73					0.23	0.04	34.99	420.91	5, 8
Nasua_nasua	0		3.69	144.91	365.00	75.06		113.15				1.93		841.82	5
Neofiber_alleni	0	0.94	2.33	9.33		28.26						0.33	20.92	95.80	5, 8
Neomys_anomalus	0		7.10	0.58		26.00						0.02	27.84	106.45	5, 8
Neophoca_cinerea	0		1.35	7147.62	539.89	265.94		542.19				26.88		1587.55	5
Neotoma_albigula	0	0.75	2.14	11.21	49.00	37.00					2.75	0.30	33.50	94.14	5, 8
Neotoma_floridana	0	0.82	3.11	14.04	83.00	34.84					0.85	0.40	37.31	175.38	5, 8

Neotoma_fuscipes	0		2.79	13.15		33.21					0.40	27.49		5, 8
Neotoma_lepida	0	0.79	2.70	9.36	60.00	32.99				0.77	0.28	33.24	94.59	5, 8
Neotoma_mexicana	0		2.59	10.40		33.21					0.31		59.82	5, 8
Neotoma_micropus	0		2.50	11.70		34.00					0.34	29.83	172.70	5, 8
Neotoma_phenax	0	0.90	2.00											5, 8
Notomys_alexis	1	1.19	4.01	2.29	32.75	32.67				0.34	0.07	30.00	76.70	1, 5, 8
Nyctalus_lasiopterus	0		1.82	5.60						0.34		59.44		5, 8
Nycticebus_coucang	0	0.92	1.12	50.47	365.00	191.09		7.00	3.90	2.59	0.26	181.21	660.82	1, 5, 8
Nyctomys_sumichrasti	0		2.00	4.33	38.00	33.98					0.13	24.85		5, 8
Ochotona_alpina	0		3.07	8.19		28.98					0.28	21.00	377.57	5, 8
Ochotona_curzoniae	0		4.39	11.20		22.50					0.50			5, 8
Ochotona_pallasi	0		7.45	7.00		25.00					0.28	19.44	365.00	5, 8
Ochotona_princeps	0		2.88	11.00	30.41	30.44				2.93	0.36	26.45	374.39	5, 8, 14
Ochrotomys_nuttalli	0	0.99	2.62	2.70	25.90	29.10				0.47	0.09	20.84		5, 8
Octodontomys_gliroides	0		2.04	20.00		104.50					0.19			5, 8
Odobenus_rosmarus	0		1.22	59090.90	821.25	357.39	591.36			238.28	165.34	729.99	2315.02	5
Odocoileus_hemionus	0		1.61	3007.49	365.00	203.49	60.00	12.60	7.20	348.21	14.78	73.49	527.12	5, 8, 12
Odocoileus_virginianus	0		1.57	2949.99	304.16	201.39	120.00	19.70	20.40	225.30	14.65	79.92	365.00	5, 8
Ondatra_zibethicus	1	0.98	6.55	21.99	30.00	27.86				4.73	0.79	27.84	198.11	5, 8
Onychomys_leucogaster	1	0.78	3.84	2.30	31.50	31.63				0.47	0.07	22.82	107.71	5, 8
Onychomys_torridus	0		3.30	2.33	29.70	28.37				0.35	0.08	19.86	56.02	5, 8
Oreamnos_americanus	0		1.40	3080.81	365.00	181.24	120.00	8.10	6.40		17.00	84.86	964.59	5, 8
Ornithorhynchus_anatinus	0		2.00		365.00	12.48						120.97	742.46	5, 8
Orycteropus_afer	0		1.10	1899.68		222.05				90.73	8.56	40.92	755.15	5, 8
Oryzomys_albigularis	0		3.40											5, 8
Oryzomys_palustris	0	0.76	4.08	3.49		24.74				1.62	0.14	11.44	49.92	5, 8
Otocyon_megalotis	1	1.02	5.50	127.10		60.00	105.00				2.12			5, 8, 9
Otolemur_crassicaudatus	0	0.93	1.14	46.57	365.00	131.04		8.00	4.80	3.64	0.36	124.62	609.86	5, 8
Ovis_aries	0		1.19	2376.24	365.00	152.54				82.87	15.58	182.50	831.62	1, 5, 8

Ovis_canadensis	0		1.16	4115.85	365.00	177.49		157.50	5.30	5.50	122.64	23.19	130.80	761.25	5, 8
Pan_paniscus	0	0.74	1.01	1399.53	1715.50	235.24	37.68	1081.31	1.10	1.00	6.24	5.95		5465.72	4, 5, 13
Panthera_onca	0		1.96	871.30	365.00	102.49		156.60				8.50	638.75	1184.16	5
Panthera_pardus	0		2.14	553.39	476.37	96.74		123.54	6.50	11.10		5.72	599.99	810.68	5, 6
Panthera_tigris	0		2.60	1313.50	821.25	150.19		118.55			81.73	8.75	570.00	1522.50	5, 8
Papio_hamadryas	0	0.56	1.01	890.00	608.33	180.00			5.10	1.50	6.62	4.94	363.96	1652.37	5, 8
Paradipus_ctenodactylus	0		3.40												5, 8
Paradoxurus_hermaphroditus	0		3.29	92.07	182.50	61.27						1.50		397.85	5, 8
Paraxerus_cepapi	0	0.96	2.02	12.75	60.50	56.87						0.22	38.39	239.99	5, 8
Paraxerus_palliatus	0	1.04	1.60	15.00	75.00	62.01						0.24	52.50		5, 8
Perodicticus_potto	0	0.97	1.09	37.16	354.05	193.00						0.19	149.15	561.58	5, 8
Perognathus_fasciatus	0		5.48			29.80									5, 8
Perognathus_flavescens	0		4.37												5, 8
Perognathus_flavus	0		3.94			26.00							29.83		5, 8
Perognathus_inornatus	0		4.00												5, 8
Perognathus_longimembris	0		4.45	1.25		22.74					0.15	0.05	18.67	125.41	5, 8
Perognathus_parvus	0		4.86	1.50		23.50						0.06	21.00	341.37	5, 8
Peromyscus_crinitus	0		3.08	2.19	26.00	25.33	6.10	23.91			0.50	0.09		71.19	4, 5, 10
Peromyscus_eremicus	1	0.97	2.52	2.51	38.50	27.35		20.88				0.09		54.07	4, 5, 8, 10
Peromyscus_gossypinus	0	0.91	3.70	2.19		23.00	4.98	22.33				0.10		49.58	4, 5, 10
Peromyscus_melanocarpus	1		2.23	4.50	33.55	30.39	4.60	25.80				0.15		91.44	5, 8
Peromyscus_mexicanus	1		2.56	4.34	33.30	30.19		31.22				0.14		53.04	4, 5, 8, 10
Peromyscus_yucatanicus	0		3.00	2.50	27.50	30.80						0.08		49.35	4, 5, 10
Petaurus_breviceps	0		1.82	0.18	311.00	16.68					0.41	0.01	118.65	320.03	5, 8
Petromus_typicus	1		1.50												5, 8
Phaner_furcifer	0		1.01			174.46									5, 8
Phenacomys_intermedius	0		4.66	2.19		22.42						0.10	30.93	40.02	5, 8
Philander_opossum	0		4.75	0.20	106.00						1.04		79.77	220.25	5, 8
Phodopus_sungorus	0		5.48	2.04	24.00	21.00					0.59	0.10	20.00		5, 8

Pithecia_monachus	1	0.81	1.02	119.85											5, 8
Pithecia_pithecia	1	0.81	1.01		448.03	161.13							113.15	1089.37	5, 8
Plagiodontia_aedium	1		1.14	105.76		136.50						0.77			5, 8
Planigale_gilesi	0		5.23	0.01	341.00	15.74					0.06	0.00	83.52	272.11	5, 8
Platanista_gangetica	0		0.97			304.16							339.47		5, 8
Poecilogale_albinucha	0		2.23	4.00	243.33	32.55		76.56				0.12		914.79	5, 8
Pongo_pygmaeus	0	0.46	1.07	1644.20	2007.50	259.82	28.81	1088.82	3.50	1.50	8.17	6.33		3318.62	4, 5, 13
Potamochoerus_porcus	0		3.00	750.00	365.00	120.00						6.25		1113.70	4, 5
Potos_flavus	0		1.11	171.84	365.00	116.80		109.13				1.47	160.00	858.41	5
Praomys_delectorum	0		3.88												5, 8
Praomys_hartwigi	0		3.49												5, 8
Praomys_jacksoni	0		3.67			35.49							30.00	74.20	5, 8
Praomys_morio	0		3.28	2.68		35.99						0.07	30.77	79.71	5, 8
Praomys_tullbergi	0	0.86	3.36	2.70	24.00	23.90					0.32	0.11	24.50	57.07	5, 8
Priodontes_maximus	0		1.50	113.00		124.24						0.91	34.80	327.59	5, 8
Procapra_gutturosa	0		1.41	2900.00	365.00	189.94						15.27	38.97	893.06	5, 8
Procavia_capensis	0		2.50	203.49	365.00	231.24					3.26	0.88	90.99	487.07	5, 8
Procyon_lotor	0		3.06	82.46	365.00	65.00		104.20	4.20	6.10	9.72	1.27	317.49	561.28	5
Proechimys_guairae	0		2.79	25.45		62.99						0.40	10.21	73.21	5, 8
Propithecus_verreauxi	0	0.91	1.02	102.65	365.00	149.77						0.69	177.83	943.94	5, 8
Psammomys_obesus	0	0.75	3.49	6.20	38.30	24.80					1.40	0.25	24.18	63.38	5, 8
Pseudomys_australis	0		3.00	4.47		31.35					0.31	0.14	25.90	183.07	5, 8
Pseudomys_desertor	0		3.00		29.75	28.96							24.49	79.32	5, 8
Pseudomys_hermannsburgensis	0		2.00			31.50							30.00	91.53	5, 8
Pteronura_brasiliensis	1		2.12	204.25	365.00	70.00		120.48				2.92			5, 8
Pudu_puda	0		1.22	780.00		210.00					38.05	3.71	54.75	229.14	5
Pygeretmus_platyurus	0		5.17												5, 8
Pygeretmus_pumilio	0		3.35			27.50									5, 8
Pygeretmus_shitkovi	0		5.49												5, 8

Rangifer_tarandus	0		2.00	5490.62	365.00	222.50		119.20	22.50	10.30	162.89	24.68	136.87	758.71	1, 5, 8, 12
Rattus_rattus	0	0.98	5.88	4.53	31.25	23.45					1.16	0.19	28.00	115.29	5, 8
Ratufa_bicolor	0		1.50	77.00	149.00	31.50						2.44	68.20		5, 8
Reithrodontomys_fulvescens	0	0.98	3.76	1.11							0.15		14.44		5, 8
Reithrodontomys_humulis	0		2.99	1.07	34.05	21.26						0.05	20.92	44.65	5, 8
Reithrodontomys_megalotis	0	1.04	4.18	1.36	27.60	23.50						0.06	20.00	87.50	5, 8
Rhinoceros_unicornis	0		1.41	61326.59	909.45	478.99						128.03	339.47	2069.22	5, 8
Rhizomys_pruinosus	0		3.00										120.75		5, 8
Rhynchocyon_chrysopygus	1		1.22	80.00	81.00	42.27					7.14	1.89	14.00		5, 8
Rhynchocyon_cirnei	1		1.93												5, 8
Rhynchocyon_petersi	1		1.93												5, 8
Romerolagus_diazi	0		2.10	25.86		39.26					2.24	0.66	28.71	180.18	5, 8
Saccostomus_campestris	0	0.94	6.75	2.59	51.80	20.84						0.12	28.97	50.56	5, 8
Saccostomus_mearnsi	0	0.78	5.75												5, 8
Saguinus_fuscicollis	1	1.04	1.83	39.18	293.50	148.00	16.57	90.10				0.26		406.61	4, 5 ,8 ,13
Saguinus_geoffroyi	1	1.04	1.97	47.84	240.90	160.00		75.00				0.30		720.00	4, 5 ,8 ,13
Saguinus_labiatus	1	1.08	1.84	40.67	299.30	144.16						0.28			4, 5, 8, 13
Saguinus_nigricollis	1	1.03	1.82	43.58	252.00			82.53			1.59				5, 8, 13
Salpingotus_crassicauda	0		2.59												5, 8
Sciurus_carolinensis	0	1.00	2.98	15.16		44.79		69.47			2.66	0.34	304.16	337.88	4, 5, 8
Sciurus_granatensis	0	0.95	1.90	9.50											4, 5
Sciurus_griseus	0	1.28	2.65			43.49								330.37	4, 5
Sciurus_niger	0	0.99	3.26	15.08		44.67		64.71				0.34		365.00	4, 5, 8
Scotinomys_teguina	0		2.46	1.11	30.95	31.20						0.04	21.00	38.97	5, 8
Setifer_setosus	0		3.24	24.70		57.63						0.43	25.22	216.55	5, 8
Sigmodon_hispidus	0	0.73	5.44	6.60		27.00					0.76	0.24	15.29	50.19	5, 8, 14
Solenodon_paradoxus	1		1.64	89.99		64.80						1.39	74.71	827.69	5, 8
Sorex_araneus	0		6.56	0.42	44.56	21.50					0.37	0.02	21.43	312.90	5, 8
Sorex_cinereus	0		6.49	0.28		18.11					0.16	0.02	19.88	147.56	5, 8

Spalax_microphthalmus			2.91											5
Spermophilus_armatus	0	0.80	5.40			24.50		21.43				21.00	420.91	4, 5
Spermophilus_beldingi	0	0.99	5.71	6.86		26.19	4.70	24.50		2.15	0.26	56.00	420.91	4, 5
Spermophilus_lateralis	0	0.84	5.18	6.09		28.42		41.99		1.89	0.21	38.63	420.91	4, 5
Spermophilus_richardsonii	0	0.60	7.59	6.24		22.80	5.10	29.33		3.37	0.27	29.26	340.29	4, 5
Spermophilus_saturatus	0	0.95	5.10	5.97								41.67		4, 5, 10
Spermophilus_tereticaudus	0	0.69	6.32	3.84		27.57		34.80			0.14	27.67	324.83	4, 5
Spermophilus_townsendii	0	0.67	8.49	3.70		22.05		32.55		1.18	0.17	27.50	413.84	4, 5
Spermophilus_tridecemlineatus	0	0.84	8.08	3.00		27.50		27.81			0.11	28.42	264.16	4, 5
Spilogale_putorius	0		5.07	12.29	243.33	32.99		55.68			0.37		196.96	5
Steatomys_pratensis	0	1.14	3.96	1.55										5, 8
Stylodipus_telum	0		3.49											5, 8
Suncus_etruscus	1		4.00	0.20		27.50				0.09	0.01	20.00		5, 8
Sus_scrofa	0		4.52	807.77	230.00	115.20	21.65	97.88		57.85	7.01		350.76	1, 4, 5, 12
Sylvilagus_aquaticus	0		3.00	55.70		37.24					1.50	20.88	154.71	5, 8
Sylvilagus_bachmani	0		3.35	27.25	29.50	27.00					1.01	16.27		5, 8
Sylvilagus_floridanus	0		4.62	34.15	30.45	27.00				3.42	1.26	22.78	106.05	5, 8
Synaptomys_cooperi	0	1.07	3.09	3.15		23.30				0.83	0.14	20.41		5, 8, 14
Tachyoryctes_splendens	0		1.41	15.00	173.00	39.01					0.38	35.07	137.21	5
Talpa_europaea	0		3.89	3.24		30.41				1.49	0.11	32.49	335.48	5, 8
Tamias_amoenus	0		4.99	2.62	365.00	30.46		41.99			0.09		350.01	5
Tamias_dorsalis	0	1.10	4.99			29.49		30.00						5, 10
Tamias_obscurus	0		3.49											5, 10
Tamias_palmeri	0		3.93	3.29		33.21		81.85		0.22	0.10			5
Tamias_ruficaudus	0		4.85											5
Tamias_sibiricus	0		5.01		103.50	34.98		29.69					352.43	5, 10
Tamias_sonomae	0		4.00					21.00						5
Tamias_townsendii	0	1.10	3.75	3.52		28.00		41.76		0.75	0.13			5, 10
Tarsius_syrichta	0	0.87	1.01	25.60		177.99					0.14	82.49		5, 8

Taxidea_taxus	0	0.97	2.76	93.49	365.00	43.80	41.99				2.13	365.00	365.00	5, 10
Tayassu_pecari	0		2.00	1000.00		158.00	13.64				6.33		684.67	4, 5
Thallomys_paedulcus	1	1.07	3.20	2.65	26.00	26.34					0.10	29.49	91.83	5, 8
Thomomys_bottae	0	0.82	4.61	3.20		23.87					0.13	35.73	201.38	5, 8
Thomomys_talpoides	0	0.95	4.86	3.15	365.00	19.00					0.17	33.12	231.94	5, 8
Thomomys_umbrinus	0	0.77	2.20											5, 8
Thryonomys_gregorianus	0	1.01	2.91			91.24								5, 8
Thryonomys_swinderianus	0	0.73	3.88	118.89		125.76				12.51	0.95	25.17	175.38	5, 8
Tremarctos_ornatus	0		1.44	320.00		215.00					1.49	173.44		5, 8
Trichosurus_caninus	0		1.02		363.00	16.20						241.95	1095.00	5, 8
Tupaia_glis	0		2.22	12.60	45.30	45.99				2.55	0.27	34.27	122.77	5, 8
Tupaia_montana	0		2.01	11.00		48.99					0.22	27.84		5, 8
Uromys_caudimaculatus	0	0.95	1.46	20.00		38.85					0.51	40.00	184.63	5, 8
Urotrichus_talpoides	0		3.49			36.05						27.79	371.23	5, 8
Ursus_arctos	0		2.24	499.99	912.50	227.56				139.73	2.20	182.50	1327.95	1, 5, 8, 12
Ursus_maritimus	0		1.66	670.48	831.67	64.66	450.00	31.00	10.20	17.40	10.37	205.17	1850.26	1, 5, 8
Vulpes_bengalensis	0	0.92	3.49	58.49	365.00	52.33					1.12			5, 8, 9
Vulpes_cana	1	0.87	2.00	29.00		57.35	44.74				0.51	150.00	380.55	5, 8, 9
Vulpes_corsac	1	0.98	5.62	63.12	365.00	54.99	54.56				1.15		1024.89	5, 8, 9
Vulpes_velox	1	0.92	4.25	39.94	365.00	53.70	47.08				0.74	150.00	470.29	5, 8, 9
Vulpes_zerda	0	0.71	2.36	28.04	365.00	51.00	65.56				0.55		294.06	5, 9
Zaedyus_pichiy	0		2.00	105.00		61.27					1.71	41.88	303.29	5, 8
Zalophus_californianus	0		1.41	6347.89	547.50	349.99	319.01			43.42	18.14		2023.55	1, 5
Zapus_princeps	0		5.04			18.11						29.04		5, 8
Zapus_trinotatus	0		5.47	0.80		21.37					0.04	30.18		5, 8
Zygodontomys_brevicauda	0	0.78	4.23	3.54	25.00	25.39				1.00	0.14	18.50	42.69	5, 8